DISEASES OF THE BLOOD

PAUL W. CLOUGH

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By PAUL W. CLOUGH, M.D.

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Harper's Medical Monographs

DISEASES OF THE BLOOD

By

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DISEASES OF THE BLOOD

CHAPTER I

INTRODUCTION

BY DISEASES of the blood are ordinarily understood those pathological conditions which are associated with abnormalities in the number or character of the blood corpuscles. Important as are the chemical changes in the plasma, these are so complex and so diversified in origin and character that a discussion of them would cover nearly the entire field of internal medicine. They will, therefore, receive no consideration except for the small group of conditions which are associated with defects in blood coagulation.

Even in this restricted sense there is, strictly speaking, no such thing as a disease of the blood. The blood is not a living tissue, but is merely a passive vehicle for the transportation of materials, chiefly food, oxygen, and the waste products of combustion. Even the blood corpuscles are not comparable to the cells of an ordinary tissue. They are very highly specialized structures which are no longer capable of reproduction, and which in general function in as passive a manner as do the fluid constituents of the blood. Abnormalities in the blood corpuscles must, therefore, be regarded as symptoms of disease elsewhere in the body.

In a limited group of conditions the disease process involves primarily the blood-forming or-

gans (hemopoietic tissues), as, for example, the leucemias (so called primary blood diseases). In a much larger number of cases some other tissue is primarily involved, and the changes in the blood are incidental and symptomatic. In some instances these changes are due to the direct action of the disease noxa on the circulating corpuscles, but more often, and more significantly, they result from a stimulation or a depression of the hemopoietic tissues by the disease process. In a study of these conditions we must always consider them as diseases or disturbances of the blood-forming organs, and not simply of the blood itself. However, a study of the blood, and more particularly of the blood corpuscles, gives information concerning the functional capacity and reacting power of the hemopoietic tissues which is often of diagnostic significance. For a proper understanding of these diseases, therefore, it is essential to be familiar with the normal appearance and function of the different types of blood cells, and with the changes in them which may be met with in disease.

There are three different kinds of blood corpuscles, all of which require careful consideration: the red corpuscles, or erythrocytes; the white corpuscles, or leu cocytes, of which at least five distinct varieties occur in normal blood; and the blood platelets, or thrombocytes.

CHAPTER II

THE ERYTHROCYTES

Morphology. These cells are circular biconcave discs, visibly thinner and paler in the center than at the periphery. They contain no nucleus, and show no definite internal structure. They are extremely elastic, and may undergo a marked temporary distortion in shape when squeezing through narrow passages. In fresh preparations they have a yellowish color, due to the hemoglobin contained in them. They consist of a relatively large proportion of hemoglobin held together by a relatively small amount of stroma, which constitutes the scanty residue of the cell after the hemoglobin has been dissolved out by distilled water. The mixture of hemoglobin and stroma forms a somewhat viscous mass, the outer layer of which seems to be denser, and serves as a functional, if not an anatomical limiting membrane.

The primary *Function* served by the red blood cells is to convey oxygen from the lungs to the tissues. Their capacity to do this depends entirely on their hemoglobin content. In the lung capillaries the oxygen tension is high, and the hemoglobin molecule combines chemically with a molecule of oxygen to form oxyhemoglobin. In the tissue fluids the oxygen tension is low, because oxygen is being consumed. As a result the oxyhemoglobin,

which is an unstable compound, dissociates; the oxygen is liberated, and the reduced hemoglobin is carried back to the lungs where the oxygenation is repeated. The direct result of a gross deficiency of red cells and hemoglobin is, therefore, oxygen starvation of the tissues, anoxemia. The carbon dioxide is carried from the tissues to the lungs in solution, and does not enter into chemical combination with the hemoglobin. However, the changes in reaction which accompany the oxygenation and reduction of hemoglobin indirectly facilitate the transportation of carbon dioxide, and also assist materially in maintaining the normal acid-base equilibrium of the body.

The *Number* of red corpuscles is determined by a direct enumeration of the cells present in a known volume of properly diluted blood. (Page 270). The average red cell count of a normal male in the third decade is slightly over 5 million cells per c.mm. Osgood[1] reports an average in 137 men of 5.39 millions, with a range of normal at least from 4.7 to 6.0 millions. These figures are somewhat higher than those reported by Haden, and some other observers. The average in normal women is about 10 per cent lower than that in men. Osgood and Haskins[2] report an average in 100 women of 4.8 millions, with a range of normal variation at least from 4.3 to 5.9 millions. The average count for other ages is not so well established. In the new-born infant there is a plethora,

[1] Osgood, E. E., *Arch. Int. Med.*, 1926, xxxvii, 685.

[2] Osgood, E. E., and Haskins, R. D., *Arch. Int. Med.*, 1927, xlix, 643.

with a count of about 6 millions. This disappears after a few days, and during infancy and childhood the count is somewhat below that in the adult, rising gradually during adolescence to the adult figure. With advancing age there is a tendency to fall slightly.

Hemoglobin. The determination of the quantity of hemoglobin, which should be the simplest and one of the most valuable of the methods of blood examination, is, on the whole, the least satisfactory, and the results as ordinarily obtained are the least accurate. This is largely due (1) to the multiplicity of methods in use, each with its own standard of normal; and (2) to failure properly to control the sources of error inherent in the instrument used, and particularly failure to keep the instrument properly standardized. If hemoglobin determinations are to be of value it is necessary to define precisely what the standard of normal (100 per cent) really means, to understand the possible errors in the method used, and to take adequate precautions to avoid them.

The logical and scientifically *accurate method of expressing the hemoglobin content* of a specimen of blood is in terms of weight; that is, as grams of hemoglobin per 100 cc. of blood. When hemoglobin estimations were first attempted there was no method available for determining this, even indirectly. For want of any better method the depth of color of a specimen of blood was compared in some form of colorimeter with that of "normal" blood, and the result was expressed in terms of the percentage of normal. This method of expression

has been retained in substantially all the practicable methods of hemoglobin estimation, despite the fact that it is scientifically illogical, and that practically it has been a source of much confusion and error. There is still no method of determining directly the hemoglobin content of blood. All available methods do this indirectly.

The most accurate method consists in determining the *oxygen combining capacity of the blood* by Van Slyke's method. It has been shown that 1 gram of pure hemoglobin will combine with 1.34 cc. of oxygen. By measuring the volume of oxygen liberated in vacuo from a measured volume of completely oxygenated blood, and by dividing this figure by 1.34, the amount of hemoglobin (in grams) in that volume of blood is obtained. This method, unfortunately, requires expert technique and special apparatus. It is of fundamental importance, however, in the accurate standardization of other types of instruments.

All practicable clinical methods are *colorimetric*. They are based on the assumption, which is substantially correct, that the quantity (that is, the weight) of hemoglobin in a specimen of blood is proportional to the intensity of its color. Blood, diluted or undiluted, is compared with some color (or colors) which is made to correspond to the color of what the designer conceives to be normal blood, and which is designated as 100 per cent. The multiplicity of methods and instruments which have been suggested is sufficient evidence that none of them is altogether satisfactory. Of the numerous instruments now on the market the writer recom-

mends the Sahli hemometer as being on the whole the most satisfactory for general use. The disadvantages of the instrument, as well as the precautions to be observed in its use are discussed in detail on page 274.

There is not as yet general agreement as to what should constitute the *normal, or 100 per cent of hemoglobin.* Strictly speaking, there can be no such thing as a rigid standard of normal. The amount of hemoglobin in normal individuals varies with sex, with age, and not improbably with race and geographic location. Many different standards have been suggested, varying from that of Haldane, 13.8 grams per 100 cc. of blood, to that of Sahli, 17.3 grams per 100 cc. The first is unquestionably too low. The latter is undoubtedly too high, and represents a maximum rather than an average normal standard. For reasons that will be clear when the subject of color index is discussed it is desirable that 100 per cent of hemoglobin correspond to the average hemoglobin content of a normal blood with a red cell count of 3 million cells per c.mm. The writer recommends the general adoption of *the standard* suggested by Haden,[3] of *15.6 grams* per 100 cc. blood. This corresponds to an oxygen combining capacity of 21 volumes per cent. This figure has already come into fairly extensive use, and is in quite close agreement with the findings of most other observers in this country.

The *average hemoglobin* of normal men in the third decade is between 15.6 and 15.8 grams per 100 cc. The range of normal variation is at least

[3] Haden, R. L., *Arch. Int. Med.*, 1923, xxxi, 766.

from 14 to 18 grams (Osgood). For women of this age it is about 10 per cent lower. Osgood and Haskins reported an average of 13.7 grams, with a range of normal variation of 12 to 16 grams. Data for other ages are relatively scanty, but the figures vary from those of a young adult in about the same proportion as does the red cell count. In normal individuals variations in hemoglobin closely parallel variations in the red cell count.

The Total Hemoglobin Content of the Body is obviously of greater significance than merely its concentration in the blood. To determine this it is necessary also to measure accurately the total volume of blood in the body. Unfortunately methods of determining blood volume are not satisfactory, and those available are hardly practicable except in a hospital, or in the hands of one who is specially trained in their application.

There are two types of methods in use for measuring the Blood Volume: (1) *The dye method*, introduced by Keith, Rowntree, and Geraghty,[4] in which a dye (such as vital red) is injected intravenously, and a few minutes later the concentration of dye in the plasma is determined colorimetrically. This method is relatively simple, and results obtained with it are generally regarded as at least sufficiently accurate for all practical purposes. By this method the normal blood volume averages about 8.8 per cent of the body weight. (2) *The carbon monoxide method*, in which a measured volume of carbon monoxide is inhaled by the patient,

[4] Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547.

Plate I.

Figure 1. Polymorphonuclear Neutrophile.

Figure 2. Polymorphonuclear Basophile.

The lobes of the nucleus are indistinct and are partly overlaid with granules. The latter vary in their size and depth of staining.

Figure 3. Polymorphonuclear Eosinophile.

The granules are large and uniform in size.

Figure 4. Clump of Normal Platelets.

Figure 5. Small Lymphocyte,

with a "wheel spoke" arrangement of the chromatin in the nucleus. There is a definite clear zone in the cytoplasm around the nucleus.

Figure 6. Small Lymphocyte,

containing azure granules. The chromatin is in large masses.

Figure 7. Large Lymphocyte,

with azure granules. The chromatin is in coarse masses, and there is an indistinct nucleolus. There is a pale zone in the cytoplasm around the nucleus.

Figures 8 and 9. Monocytes.

The cytoplasm is grayish blue in color. There is a fine lilac granulation which is abundant but does not stuff the cell as does that of the granulocytes. The nuclear chromatin is arranged in a fine network as contrasted with the coarse skeins of the granulocytes and the dense masses of the lymphocytes. There are no nucleoli. No. 9 is the "transitional" of Ehrlich.

Figure 10. Plasma Cell.

The cytoplasm is abundant, basophilic, with two vacuoles, and a small pale perinuclear zone. The chromatin masses are coarse and show the "wheel spoke" arrangement.

Figure 11. "Giant" Platelet,

from a case of pernicious anemia.

Figure 12. NEUTROPHILIC METAMYELOCYTE,

with the band-shaped nucleus.

Figure 13. NEUTROPHILIC MYELOCYTE,

with oval nucleus and practically mature granulation.

Figure 14. EARLY TYPE OF MYELOCYTE,

with sparse bluish undifferentiated granules, and cytoplasm diffusely basophilic except for a narrow acidophilic zone to the left of the nucleus. The nuclear structure is fine, and there are two nucleoli.

Figure 15. MYELOBLAST.

There is no distinct nuclear membrane, the background of the nucleus is very finely stippled, and there are five nucleoli. The cytoplasm is deeply basophilic, is free from granules, and shows no pale perinuclear zone.

Figure 16. VERY EARLY PREMYELOCYTE.

Essentially identical with the myeloblast except for the few reddish rod-shaped structures (Auer bodies) and bluish granules.

Figure 17. LYMPHOBLAST.

The cytoplasm is deeply basophilic, with a perinuclear pale zone. There is a definite condensation of chromatin in the nuclear membrane and around the two nucleoli. The chromatin is appreciably coarser than in the myeloblast.

Figure 18. GIANT NEUTROPHILIC LEUCOCYTE,

with markedly segmented nucleus, from a case of pernicious anemia.

Figure 19. POLYMORPHONUCLEAR NEUTROPHILE,

from a patient with an acute infection showing toxic degenerative changes. The nucleus is swollen, with loss of the finer markings. The cytoplasm is vacuolated, and the granules are sparse, and variable and abnormal in size and tint.

Figure 20. RIEDER CELL,

or large lymphocyte with basophilic cytoplasm and a convoluted or lobulated nucleus.

Figure 21. MEGALOBLAST

of a very early type. The structure of the nuclear chromatin is fine, and there are two nucleoli. The cytoplasm is primitive and has an opaque greenish blue color, but shows a slight tendency to become acidophilic near the lower pole of the nucleus. The margins of the cell look thick.

Figure 22. MEGALOBLAST

of a late type, with acidophilic cytoplasm and a nucleus which is becoming pycnotic and has undergone a curious type of fragmentation.

Figure 23. MEGALOBLAST

of an early type, with basophilic greenish cytoplasm, and a nucleus with a relatively fine chromatin network, but somewhat coarser than in Figure 21.

Figure 24. IMMATURE NORMOBLAST,

with faintly basophilic cytoplasm, and characteristic wheel spoke arrangement of the nuclear chromatin.

Figure 25. MEGALOBLAST,

with the nucleus undergoing mitotic division. There is a fine basophilic stippling of the cytoplasm. These four megaloblasts were drawn from the blood of an advanced case of pernicious anemia.

Figure 26. RETICULOCYTE,

"vitally stained" with cresyl blue.

Figure 27. NORMOBLAST,

with a pycnotic nucleus and fine basophilic stippling.

Figure 28. RED CELL,

with a large nuclear fragment and fine "chromatin dust."

Figure 29. RED CELL,

showing basophilic stippling and a Howell-Jolly body.

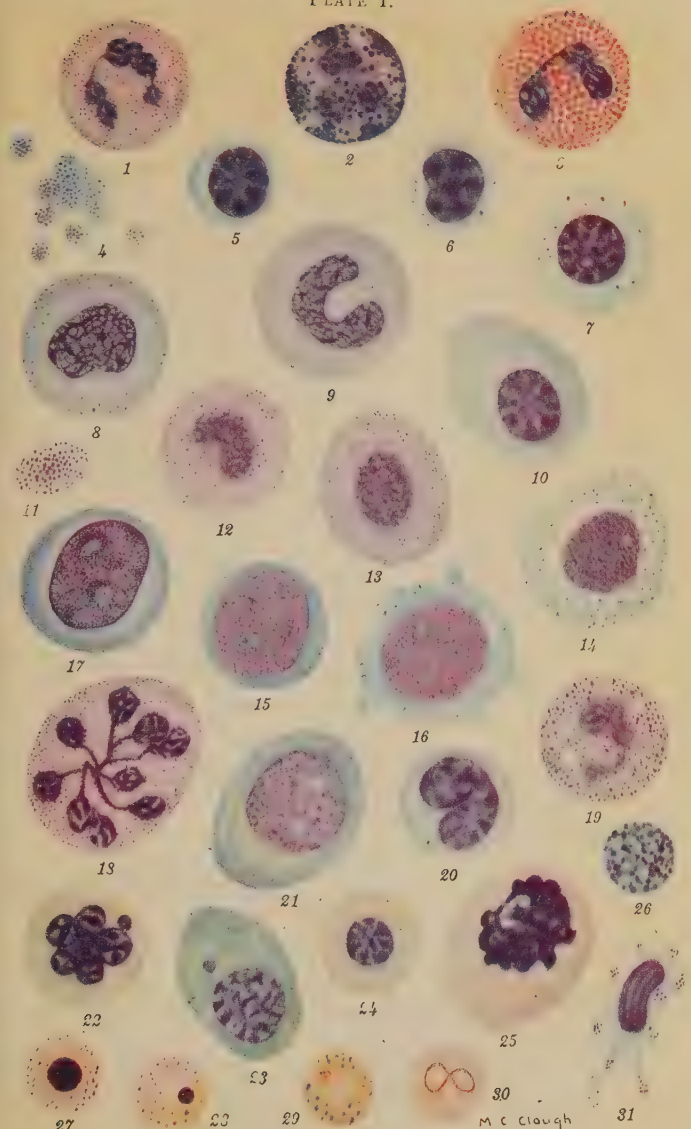
Figure 30. RED CELL,

showing a Cabot ring. The cytoplasm is diffusely basophilic.

Figure 31. MEGAKARYOCYTE,

from a case of pernicious anemia. The cytoplasm is sky blue, with small clumps of bright red granules, identical in appearance with those of the platelets. The nucleus may be round or oval rather than elongated as in this cell. Stained with Wilson's stain. Magnified 1200 diameters.

PLATE I.



TYPES OF NORMAL AND PATHOLOGICAL BLOOD CELLS

and the concentration of the gas in the blood is measured a few minutes later. This method is regarded as scientifically the more accurate, at least for the study of red cells and hemoglobin, but it requires a high degree of technical skill. By this method the average normal blood volume is about 5.6 per cent of the body weight.

The difference in the results obtained by the two methods is due to the fact that the carbon monoxide is all carried by the hemoglobin of the red corpuscles, while the dye, diffused throughout the plasma, can penetrate into partly collapsed capillaries and narrow spaces into which the cells cannot enter. Both methods measure only that part of the blood which is in active circulation, and fail to take account of the blood which may be stored in the spleen pulp, or in other stagnant areas of the circulatory system.

Color Index. The color index of a given blood represents the relative amount of hemoglobin per individual red blood cell, as compared with that of normal blood. It is independent of their volume. It is determined simply by dividing the percentage of hemoglobin by the red count, expressed as percentage of the normal count. For purposes of color index determination the normal red count is assumed to be 5 millions, and the normal hemoglobin standard recommended (15.6 grams per 100 cc. = 100 per cent) is the average amount found in normal blood having a count of 5 million cells per c.mm. Expressed more simply (if this hemoglobin standard is used), one multiplies the first two digits in the figure expressing the red cell count by two, and

divides the percentage of hemoglobin by this product. In the theoretically normal blood one divides 100, the hemoglobin percentage, by twice 50, the first two digits in the red cell count (5,000,000), and obtains the quotient 1.0, which by definition is the color index of average normal blood. Figures from 0.85 to 1.15 are within the range of normal variation. The color index, if accurately determined, is of great diagnostic value in the differentiation of anemias. In primary pernicious anemia (and a few other conditions) it is nearly always higher than 1.0, while in most cases of secondary anemia it is below 1.0.

Volume Index. The volume index of a given blood represents the relative volume of the individual red blood cells, as compared with those of normal blood. It is independent of their hemoglobin content. To determine this one must make a red cell count and determine accurately the volume of packed red cells, which is obtained by centrifugalizing to a constant volume the cells from a measured volume of blood. The percentage of the total blood volume which is made up of the packed red cells constitutes the cell volume per cent. For purposes of volume index determination the normal volume per cent may be defined as the average volume per cent of red cells obtained from normal blood having a red cell count of 5 millions per c.mm. The normal figure, according to Haden, is 46 per cent. Osgood more recently has reported 42 per cent as normal. As errors (due to inadequate sedimentation) are likely to give too high readings in routine determinations, it is safer for the pur-

pose of calculating the volume index to use the higher figure. The latter corresponds more closely with the writer's personal experience.

To calculate the volume index one divides the observed volume per cent of the blood examined, by the normal volume per cent, 46. The quotient becomes the numerator of a fraction, of which the denominator is the red cell count of the blood in question, expressed as the percentage of the normal count; that is, twice the first two digits of the red cell count. The average normal volume index is (by definition) 1.0, and the range of normal variation is 0.85 to 1.15, usually 0.9 to 1.1. In case of a pathological blood one may find a red cell count of 2.5 millions, and a volume percentage of red cells of 32 per cent—32 divided by 46, the normal volume per cent, gives 70 per cent. The red cell count per cent is 2 times 25, the first two digits in the red count: $70/50 = 1.40$, which is the volume index of this blood.

The volume index is of great diagnostic value in the recognition of pernicious anemia, as it is almost invariably increased in this disease, more regularly and more markedly than is the color index, and is rarely increased in any other condition. In secondary anemia it is usually diminished, often markedly so.

Saturation Index is a term introduced by Haden to express the relative amount of hemoglobin per unit volume of red blood cells as compared with normal blood, and is independent of their number. It may be calculated by dividing the color index by the volume index, and in normal blood is by defini-

tion 1.0. It has not thus far been found increased in any condition. That is, the substance of the normal red cell is completely saturated with hemoglobin, and supersaturation (probably) never occurs. In pathological conditions it is often moderately reduced. It may be more markedly reduced in severe, long-standing secondary anemias, especially those following chronic hemorrhage. Thus far it has attained no great value in differential diagnosis.

IMMATURE ERYTHROCYTES

The recognition of immature red blood cells is of great diagnostic importance because their presence in the circulating blood is believed to indicate an accelerated formation of new cells in response to a strong stimulus. They are regarded as hurried into the circulation before development is completed in order to meet a pressing need for cells. The cytoplasmic changes in the red cells which are believed to indicate immaturity require staining methods for their demonstration. In normal blood stained by Wright's or Wilson's stain the red cells, almost without exception, are stained a pinkish buff or brown tint. If immature cells are present, a limited number of the cells will show a more or less marked bluish tingeing of the cytoplasm. If all the cells are bluish the preparation is poorly stained. The bluish staining may be diffused throughout the cell, or it may be in the form of dark blue granules in a cytoplasm which may show the normal buff color, or be diffusely bluish. The condition is spoken of as *diffuse basophilia*, or *polychromatophilia*, when the entire cytoplasm of the cell is

bluish, and as *punctate basophilia*, or *stippling* when bluish granules are present. These granules vary greatly in size and number as well as in depth of color. The coarser ones may show an irregular jagged outline. They are not visible in fresh preparations.

There is little doubt that diffuse basophilia is a sign of immaturity. Such cells are abundant in the marrow, and in the blood of the fetus and infant. There is dispute as to whether basophilic granulation is necessarily a sign of immaturity, or whether it may be due to degenerative changes in the cells. In favor of the latter view is the extraordinary promptness with which it may appear after exposure to lead in the absence of any anemia, and the usual failure to find it in normal marrow. However, these stippled cells are almost invariably accompanied by diffusely basophilic cells, and are usually outnumbered by them. The consensus of opinion is that as a rule basophilic stippling does indicate immaturity, but is the result of a pathological (degenerative) change in the cell. Basophilic cells of either type may occur in any form of anemia in which there is active red cell formation.

Reticulated Red Blood Cells (Reticulocytes). These cells are of great diagnostic importance. They require a "vital" staining method for their demonstration; that is, the stain must be applied to the fresh blood immediately, before coagulation or drying can occur. In preparations stained with brilliant cresyl blue by the method outlined (page 281), a limited number of the cells will show small bluish staining granules or filaments. There may be only

a single granule in the cell, or there may be many such granules connected with one another by thin bluish staining strands. These cells are called skein cells, and are conspicuous objects. They are of especial significance as they are believed to be more immature than those cells with a few granules only. To pick out the latter requires close scrutiny and a well-stained preparation. These granules are quite different from the basophilic granules sometimes seen in films stained with Wilson's stain, although both are often demonstrable in the same blood. The number of cells showing reticulations is much greater than the number showing basophilic granulations, and reticulated cells may be fairly numerous in blood which contains no stippled cells.

A few such cells are present in normal blood, from 0.1 to 0.5 per cent. They may be increased in any form of anemia in which there is active regeneration of red cells, and they are believed to be the earliest and most dependable sign of such activity. Reticulated cells are not infrequently increased to 10 per cent or more. They are increased early in the successful treatment of pernicious anemia by liver feeding, and are the first sign of its effectiveness. They are especially numerous in familial hemolytic jaundice, where they may constitute from 20 to 50 per cent of the red cells.

NUCLEATED ERYTHROCYTES

(a) *"Mature" Normoblasts* are about the size of a normal red cell, or they may be a little larger. The cytoplasm may take the normal buff tint with Wilson's stain, but quite often is more or less dif-

fusely basophilic, and may show basophilic granules. The nucleus is about half the diameter of the cell, it stains a homogeneous deep purplish black, and shows no trace of internal structure. It is spoken of as pycnotic. Not infrequently such a nucleus may be notched, cleft, or irregularly lobulated or fragmented, and occasionally a cell may be found containing only a small fragment of such a nucleus. These normoblasts are the immediate precursors of the mature red cells, but in normal conditions do not enter the circulation until they have lost their nuclei. The nucleus is disposed of, in some cases possibly by direct extrusion, but it is generally believed, usually by disintegration and lysis within the cell. The lobulation above mentioned is probably a pathological form of this process. Normoblasts may appear, usually in small numbers only, in any form of anemia in which there is active blood regeneration.

(b) *"Immature" Normoblasts* tend to be somewhat larger than the normal red cell, and more often show a basophilic cytoplasm. The essential difference is in the nucleus which is not pycnotic, but shows definite internal structure. The chromatin masses are coarse, often wedge-shaped with the base outward, and are separated from one another by clear areas which radiate outward from the center of the nucleus, giving the chromatin the characteristic "wheel-spoke" arrangement.

(c) *Macroblast* is a term which has been applied to an early type of immature normoblast. These cells are fairly large, usually with a basophilic cytoplasm and a large nucleus with a somewhat finer

chromatin structure than in the preceding types. They show the wheel-spoke arrangement of the chromatin more or less definitely. These cells are believed by some (Naegeli, etc.) to be the earliest precursor of the mature erythrocyte in the normal adult, and are sharply differentiated by these writers from the megaloblasts on the basis of their nuclear structure. Such cells have been classed as megaloblasts by many other writers, and this fact explains much of the confusion and dispute as to conditions in which "megaloblasts" may be found.

(d) *Megaloblasts* are characterized by their large size, and by their distinctive type of nucleus. They are usually from 10 to 20 microns in diameter, but may be even larger. The cytoplasm may take a buff tint with Wilson's stain, but is usually somewhat basophilic, and may contain basophilic granules. In some cases it stains so deeply blue that it gives scarcely any suggestion of containing hemoglobin, but more often the blue has a distinct greenish tinge. The cytoplasm looks waxy and homogeneous, and gives the impression of being uniformly thick out to the extreme margin of the cell, while the white cells with which megaloblasts might be confused have margins which look thin and somewhat irregular. The nucleus is about the size of a normal red cell, is usually central, and has no clear perinuclear area. It has a distinct nuclear membrane and a well-defined chromatin network. This is made up of numerous interlacing strands bearing chromatin masses which in the youngest forms are quite fine, and in somewhat older cells are coarser, but finer and distinctly different from those in the types of

normoblasts above described. Once learned, these cells can be recognized easily, as their appearance is usually quite distinctive. Their occurrence in the blood indicates a profound disturbance of bone marrow function. They are found chiefly in pernicious anemia, especially during acute exacerbations of the disease. Rarely, however, megaloblasts, or cells practically indistinguishable from them, may appear in severe anemias of other character, especially in children, in myeloid leucemia, and in malignant disease of the bone marrow. They are present in normal fetal blood, and their appearance in adult blood is generally regarded as a reversion (by some, a perversion) to the embryonic type of blood formation.

According to this strict interpretation of the term "megaloblast," which is held by many hematologists, a megaloblast is not a precursor of the normoblast, but is a cell of an entirely different order. It does not develop through a normoblastic stage into a normal erythrocyte ("normocyte"), but, after loss of its nucleus, into a definitely pathological type of erythrocyte, a *megalocyte*. Its nucleus becomes pycnotic, and is eventually disposed of by a process of solution and fragmentation similar to that in case of the normoblast. The megalocyte and these intermediate stages in its maturation are distinguished from the corresponding stages in the development of the normoblast by the much larger size of the cell. The primitive megaloblasts may exceptionally enter the circulation while undergoing mitotic division (Plate II). The old term "intermediate," which includes both immature normoblasts, and

megaloblasts with more or less pycnotic nuclei, should be discarded.

Nuclear Particles in the red cells are inclusions which take a bright purplish red color with Wilson's stain. They may occur as fine dots, often clustered around the periphery of the cell, and in this form have been described as *chromatin dust*. When occurring as medium to moderately coarse granules they are called *Howell-Jolly bodies*. They may also appear as slender bands which may be ring-shaped or variously convoluted, *Cabot's rings*. These structures are generally believed to be remnants of the nucleus, or of the nuclear membrane. However, not infrequently, a single plump granule may be seen in a cell with an apparently intact nucleus. They have an entirely different color from that of the basophilic granules previously described. They occur much less frequently and in smaller numbers. The cells containing them often show diffuse basophilia. They may occur in any condition in which nucleated red cells may be expected, are often in association with them, and have the same diagnostic significance. They are almost constantly found in the blood after splenectomy, often in considerable number, even in the absence of anemia, and may persist for a year or more after operation.

Pathological Changes in the size, shape, and depth of color of the red cells occur as a result of disordered and imperfect blood formation. The normal red cell has a perfectly circular outline. Cells which show distortions in shape are spoken of as *poikilocytes*. Some of these distortions are artefacts pro-

duced in making the films, but in pathological conditions cells showing malformations may be present in the circulation. Such cells may be oval, elongated, sausage-shaped, or like a battledore or dumb-bell. They may occur in any severe anemia, but are especially numerous in pernicious anemia. A special form of poikilocyte, an oat-shaped or sickle-shaped cell, occurs in the sickle cell anemia of negroes, and will be described in detail later.

The average diameter of the normal red cell is about 7.5 microns, and there is surprisingly little variation from this figure in perfectly normal blood. Seventy per cent of the cells are within about 1/2 micron of this average figure. An occasional cell may be as low as 5 or as high as 10 microns. In pathological conditions the red cells may vary much more markedly in size. This condition is spoken of as *anisocytosis*. Small cells under 5 microns in diameter are called microcytes; large cells over 10 microns, macrocytes; and huge cells, megalocytes or gigantocytes. Anisocytosis may be met with in any type of anemia of notable degree, but is particularly marked in pernicious anemia. Most of the microcytes and some of the poikilocytes are probably portions of fragmented cells.

Apart from abnormal diversity of size, the average diameter of the red cells may be abnormally small or abnormally large. Anemias characterized by red cells of small average diameter are referred to as *microcytic anemias*, such as the secondary anemia following hemorrhage. Those with cells of large diameter are called *macrocytic*, or

megalocytic, anemias. This "macrocytosis" is particularly marked in pernicious anemia.

The depth of color of the individual cells in normal blood is nearly as constant as is their diameter. It depends on their hemoglobin content, and in the main is proportional to their thickness. It can be observed either in fresh or stained preparations. Any notable variation in the depth of color of the individual cells is spoken of as *anisochromia.* The cells may be abnormally dark or abnormally pale. Pallor of the cells ("achromia") may in some cases be due to a diminished concentration of hemoglobin in the substance of the cell (the saturation index will then be low), but is usually due mainly to abnormal thinness of the cell. Markedly thin pale cells are characteristic of chlorosis and some types of secondary anemia, especially that following hemorrhage. Abnormally dark cells owe their excess of color entirely to their thickness. There is no evidence that the concentration of hemoglobin in the cell is ever increased, nor is there evidence that there may be any physical or chemical alteration of the hemoglobin which changes its color or staining reaction. Some thick dark cells may be found in almost any type of anemia, but a predominance of such cells is a striking feature of pernicious anemia and other forms of megalocytic anemia.

CHAPTER III

THE LEUCOCYTES

THE leucocytes, or white corpuscles, do not constitute a single clear cut cell type, as do the erythrocytes, but are made up of three distinct groups of cells which differ from one another fundamentally in origin and function as well as in structure. These are the granular leucocytes, the lymphocytes, and the monocytes.[1]

In the enumeration of the leucocytes all three types of cells are included in the total count. The average normal white cell count is about 7,000 per c.mm. The range of normal variation is about 5,000 to 10,000 per c.mm. The variations in the blood of the same normal individual from day to day, and even at different hours on the same day, may be as great as this.

The GRANULAR LEUCOCYTES are so named because these cells contain definite and conspicuous granulations which are visible in fresh preparations, and, therefore, are not dependent for their demonstration on possible artefacts of fixation and staining. They are sometimes called myeloid leucocytes, because of their undisputed origin from the bone marrow, or more simply, granulocytes. There are three varieties of granulocytes which are named,

[1] Naegeli, O., *Blutkrankheiten und Blutdiagnostik*, 4th Ed., 1923.

from the staining reaction of their granules, neutrophiles, eosinophiles (acidophiles), and basophiles. The mature cell forms met with normally in the circulating blood are usually further designated as polymorphonuclear, because of the shape of the nucleus, to distinguish them from immature forms with round or oval nuclei, which may appear in the blood in certain pathological conditions.

The *Polymorphonuclear Neutrophiles* constitute from 60 to 65 per cent of the leucocytes in normal blood. The absolute number per c.mm. would, therefore, be on the average about 4,800, with a range of variation from about 3,000 to 6,000 per c.mm. In films stained with Wilson's (or Wright's) stain the nucleus takes a deep purplish-blue color, and is vesicular; that is, it shows a definite nuclear membrane, and a deeply staining, rather coarse chromatin network. The nucleus is usually divided irregularly into lobes, from two to five in number, which are connected with one another by thin strands of chromatin, but it is sometimes ribbon-shaped and convoluted. There are no nucleoli. The cytoplasm shows a faint pink background, and is filled with nearly uniform fine granules which take a pinkish, lilac, or violet-pink color. In poorly stained films it may be difficult to distinguish individual granules. In overstained films they may be purplish or bluish.

In fresh preparations they are easily recognized by the shape of the nucleus and the numerous fine refractile granules. On a warmed stage they are actively ameboid. In a quiescent state they are approximately spherical, but when in motion they pro-

ject slender pseudopodia, which may have a length of ten times that of the diameter of the resting cell. The entire cell may be stretched out into a slender ribbon-shaped structure small enough to enable the leucocyte to squeeze through minute interstices between the endothelial cells and to "wander" about in the spaces of the connective tissue of the various organs. This ameboid activity also makes it possible for the cells to engulf minute foreign bodies, such as bacteria or pigment granules, with which they may come in contact. That is, they are phagocytes, and because chiefly bacteria and other small objects are engulfed by them, they are commonly known as microphages.

These cells possess a variety of oxidative and proteolytic ferments in their cytoplasm, and as a result of such ferment activity, engulfed bacteria and other "digestible" objects undergo solution within the cell. Substances which resist such digestion are removed from the circulation, and are eventually deposited in the marrow, spleen, or other organs, or are otherwise disposed of. This phagocytosis and intracellular destruction of bacteria is a manifestation of one of the important functions of these leucocytes, namely, the power to combat infections. This activity is manifested especially toward the pyogenic cocci (staphylococcus, streptococcus, pneumococcus, gonococcus, etc.). Other species of microorganisms such as the tubercle bacillus may be little affected by them.

This function of the neutrophilic leucocytes is especially stressed because it is easily susceptible to direct proof—the whole process can be seen and

watched. However, there is no doubt that these cells possess other functions that may be of equal importance to the body as a whole. These other functions are not as well established by experimental evidence and are much less clearly understood. Thus, these cells or their precursors in the bone marrow may be a source of the antitoxins and antibacterial substances of the plasm. They may be a source of some of the ferments of the blood. The proteolytic ("autolytic") ferments they contain make possible the rapid resolution of inflammatory exudates seen most strikingly in the pneumonic lung after crisis. It has been claimed that they may take up from the plasma, and presumably render innocuous, certain substances which are dissolved in it, such as toxins and colloidal metals. Finally, a great number of these leucocytes (and lymphocytes) are lost to the body ("excreted") daily through the mucous membranes, especially of the respiratory passages and digestive tract, and they undoubtedly carry with them and remove from the tissues considerable quantities of undesirable particulate matter, and conceivably of substances in solution as well. The importance of these leucocytes as phagocytes and scavengers is well established.

The *Polymorphonuclear Eosinophiles* make up from 0.5 to 3 per cent of the leucocytes in normal blood. The absolute number varies from about 30 to a maximum of 250 per c.mm. In stained films the nucleus is similar to that of the preceding type, with a tendency to be larger, paler, and less lobulated. The cytoplasm is densely filled with uniform coarse granules which stain a bright crimson-red

color. In poorly stained films the granules may be brownish, or pale red, and may be distinguishable from the neutrophilic granules more by their coarseness and striking uniformity in size than by their color. In a fresh preparation the eosinophiles are conspicuous because of the coarseness and high refractility of the granules. On a warm stage they are ameboid, although less actively so than the neutrophiles. Phagocytosis is occasionally observed but is rare. The function of these cells is not definitely known. Because their numbers are increased in certain diseases associated with protein hypersensitiveness, and in infections with animal parasites, it may be inferred that they serve a special function in bringing about the disintegration and removal of foreign protein from the tissues, and in combating animal parasites.

The *Polymorphonuclear Basophiles* (mast cells) constitute 0.5 per cent or less of the leucocytes. The nucleus is similar to that of the preceding types of cells, but tends to stain less deeply, and it is often difficult to make out the lobulation. The cytoplasm is more or less filled with coarse purplish or bluish black granules. These often vary considerably in size and depth of staining, and may be rather sparse. These cells are not often seen in fresh preparations unless carefully searched for. The granules are less refractile than those of the eosinophiles, and are correspondingly less conspicuous. The coarseness of the granules distinguishes them from the neutrophiles. Nothing definite is known as to the function of these cells. Some authorities regard them as degenerating structures.

The Lymphocytes make up from 20 to 25 per cent of the leucocytes. The absolute number is between 1,500 and 2,000 per c.mm. Some place the upper normal limit at 30 per cent, and the absolute number at 2,500.

The nucleus is relatively large, is round or oval, and is often notched. In stained films it is deep purplish blue, shows a dense nuclear membrane, and is specially characterized by the coarse blotchy masses of basichromatin. It may contain one or two nucleoli, rarely visible except in crushed cells, characterized by a round or oval shape, a clear-cut outline, and a pale sky blue color, much paler than the mass of the nucleus. The cytoplasm is usually scanty, homogeneous, sky blue to deep blue in color, and is usually paler than the nucleus. The nucleus is as a rule eccentric, and there is usually a thin crescentic, clear (unstained) zone next to the nucleus, between it and the bulk of the cytoplasm. No granules are visible in fresh preparations but in about 1/3 of the cells in stained films there are a few reddish violet "azure" granules. These vary much in number, size, and shape, and often appear jagged and irregular. Occasionally a cell may contain a large number of them.

Lymphocytes vary greatly in size. The typical small lymphocyte is but little larger than a red cell, while the large forms may have a diameter two to four times as great. The diameter of the average polymorphonuclear neutrophile is taken as the arbitrary dividing line between the "large" and "small" lymphocytes. The small lymphocyte is

believed to be the more mature form, and makes up a large majority of these cells in the normal adult. The large forms are believed to be more or less immature cells, and are present in larger number in children, and often in adults in the presence of infections or other conditions which cause a stimulation of the leucopoietic tissues. The large lymphocytes possess relatively much more abundant cytoplasm, and a larger nucleus, which usually takes a paler stain and shows finer chromatin masses, although the latter are obviously coarser than in other types of cells with which the lymphocytes are likely to be confused—an important point in their identification. A large lymphocyte with a mature type of nucleus (coarse chromatin masses) is not to be regarded as an immature cell. Little is gained by separating "large" from "small" lymphocytes in the differential count.

These cells are readily recognized in fresh preparations by the shape of the nucleus and the absence of granules, although large lymphocytes may be confused with monocytes. Lymphocytes are ameboid on a warmed stage. Their activity is quite different from that of other types of leucocytes: it is relatively sluggish, but is quite sufficient to explain the capacity of these cells to "wander" into the tissues. They are not phagocytic and do not contain oxydative or proteolytic ferments. Little is definitely known as to their function. There is strong indirect evidence that they play an important rôle in the defense against infection, particularly against tuberculosis and other relatively chronic infections. How this activity is carried out is not known.

The Monocytes make up from 6 to 8 per cent of the total leucocytes. Their absolute number is from 300 to 600 per c.mm. These are the largest cells of normal blood. The nucleus is large, round, oval, or, in the older cells ("transitionals" of Ehrlich), more or less notched, horseshoe-shaped or convoluted. It takes a pale bluish violet stain, and has a fine characteristically reticulated chromatin network, with slight thickenings at the points of intersection of the threads. In the older cells the chromatin network is coarser and stains more deeply. The cytoplasm is abundant, pale, grayish blue, and has the appearance of great thinness. In well-stained films there are numerous fine lilac or reddish blue granules, shown most characteristically with the Giemsa stain, and regarded by Naegeli as a specific type of granulation. In overstained preparations (Wilson's stain) the granules may look coarse and deep purplish in color. These cells must be differentiated from lymphocytes on the basis of structural differences and not of size. Nothing is gained by separating "large mononuclears" from "transitionals" in the differential count.

In fresh preparations these cells are readily recognized by their size, the shape of the nucleus, and the absence of granules. On a warmed stage they are actively ameboid and are phagocytic. They occasionally engulf bacteria, but in the main they take up damaged red cells or cell fragments, disintegrating leucocytes and similar detritus. For this reason they are classed as macrophages. In all probability most of the monocytes of the blood are

different from (though closely related to) the clasmatocytes, the large phagocytic (reticulo-endothelial) cells of the connective tissue. According to Sabin [2] clasmatocytes are normally present in the circulating blood. They can be distinguished from monocytes proper only by special supravital methods of staining. This distinction is not as yet of any practical clinical significance, and in this book the term monocyte is used to include both varieties of cells. The chief function of the monocytes in the circulation is probably to act as scavengers. They also play an important rôle in the defense against tuberculosis (Sabin *et al.*), and probably other infections.

IMMATURE LEUCOCYTES

In the blood of the normal adult practically all the leucocytes present are fully developed mature cells. Some mechanism effectively prevents the passage into the blood of cells before they have completed their cycle of development. In pathological conditions this mechanism may be disturbed and immature cells may appear. These cells are not in themselves necessarily pathological, but are the normal ancestors of the mature blood cells. It is the phenomenon of their appearance in the blood that is pathological. In general, the more numerous these cells and the greater the degree of their immaturity, the more serious the disturbance of the hemopoietic tissues.

[2] Sabin, F. R., and Doan, C. A., *Jour. Exper. Med.*, 1926, xliii, 823.

IMMATURE MYELOID CELLS

The Myelocytes are the immediate precursors of the mature granular leucocytes. The more nearly mature myelocytes, like the granular leucocytes, are divided into three groups, according to the staining properties of their granules: neutrophilic, eosinophilic, and basophilic. They differ from the corresponding types of mature cells chiefly in the shape of the nucleus, which is round or oval and which shows little or no indentation. The nucleus also tends to take a paler stain and to show a finer chromatin network than does that of the mature cell. In fresh preparations they are not phagocytic, and show little or no motility. The cell seems to have the consistency of a relatively rigid gel, rather than the fluidity of the mature cells. Every transition may be found from the cell with a perfectly round nucleus to a fully mature cell with a lobulated nucleus. The degree of lobulation may usually be taken as a rough indication of the maturity of the cell. To those intermediate forms which are difficult to classify the term "transitional myelocyte" or "metamyelocyte" is sometimes given.

The less mature myelocytes differ in the following points: The cytoplasm tends to take a bluish rather than a pinkish stain, and in extremely young cells it may be quite a deep blue. The granules do not take a distinctively lilac, red, or blue-black stain, but are undifferentiated and all take a bluish color. The granules tend to be fewer in number, and in the earliest forms there may be but few, or only a single granule in a cell. The cells tend to be

larger and to have a larger nucleus. The nucleus tends to take a fainter stain and shows a finer chromatin network, like that of the myeloblasts.

The MYELOBLASTS are the precursors of the myelocytes, and are the earliest recognizable forebears of the mature granular leucocytes. The cell is usually large, although small forms occur. It has a relatively large, usually eccentric nucleus which stains a rather light reddish purple color. It looks relatively homogeneous and velvety at first glance, but on close inspection is seen to contain an abundant fine chromatin network. There is no distinct nuclear membrane. There are usually several (1 to 5 or more) nucleoli which are pale sky blue in color, and vary much in size and shape. They have no distinct nucleolar membrane, and look like irregular lacunæ in the chromatin network, rather than such discrete spherical structures as may be seen, one or two in number, in the nucleus of the lymphocyte. While the nucleus of these early types of cells is usually round or oval it is occasionally indented or convoluted, or coarsely lobulated.

The cytoplasm may be moderately abundant, but more often it is rather scanty and takes a deep blue stain. At least a narrow rim of cytoplasm may be seen entirely surrounding the nucleus, and there is no perinuclear clear area. By definition the myeloblast contains no granules. A similar cell that contains granules is called a myelocyte. Every transition may be found between myeloblasts and typical myelocytes. To the early forms of myelocytes containing but a few granules the term promyelocyte is sometimes given.

Typical myeloblasts have a fairly distinctive appearance, and once learned they can be recognized without much difficulty. However, myeloblasts may vary markedly from type in various ways, and not rarely small forms are met with, having a denser, coarser nuclear structure, which, considered individually, could not possibly be differentiated from lymphocytes.

IMMATURE LYMPHOCYTES

Immature lymphocytes, or lymphoblasts, are usually large cells with abundant cytoplasm and large nuclei. The cytoplasm may stain with varying intensity from a pale blue to an intense deep blue. Azure granules and a pale perinuclear area may be made out in some of them. The nucleus stains less intensely than that of the small lymphocyte, and shows a finer chromatin structure, but this is usually appreciably coarser than in the myeloblast, and there is a well-defined, relatively dense nuclear membrane. One or two (rarely three) nucleoli may be present, similar to those in the myeloblast, but typically more uniform in size, more nearly spherical, and with sharper outlines, suggesting a distinct nucleolar membrane.

The term "Rieder Cell" has been applied to lymphoblasts which have a deeply indented or convoluted nucleus. It is sometimes less appropriately also applied to myeloblasts with such nuclei.

Plasma Cells are regarded by most observers as lymphatic in origin. They are not to be regarded as necessarily precursors, but often as collaterals of the ordinary lymphocytes. They are usually large

cells with abundant strongly basophilic cytoplasm, which often contains vacuoles and frequently azure granules. There is often a perinuclear pale area. The nucleus is usually eccentric and typically contains coarse wedge-shaped chromatin masses which tend to assume a radiating wheel-spoke arrangement. In some cells the chromatin may be in fine masses and evenly distributed. An occasional plasma cell may be found in normal blood. A few may be present in any condition in which lymphocytes are increased.

Tuerk's Irritation Forms are pathological types of leucocytes which are regarded by Naegeli as plasma cells or pathological lymphoblasts because of the structure of the nucleus. They are large cells, typically with deeply basophilic cytoplasm which is often vacuolated and contains no granules. They have been described in a variety of conditions in which there was an intense stimulus to the leucopoietic tissues.

Immature Monocytes tend to show scanty granulation, and to have round, pale staining nuclei with a fine reticular chromatin network. Ancestral forms, if they occur in the blood, cannot be recognized with certainty.

Pathological Leucocytes

This term is applied to individual cells which vary markedly in some feature from the normal cell of their type. They may be immature cells, but a cell is not "pathological" because it is immature. The abnormality may be the result of some

perversion of development, or it may be due to some direct toxic injury to the cell.

Abnormalities in development are diverse in character, and may involve either the nucleus or cytoplasm. The cell may be abnormally large or small. The nucleus may be of unusual size. It may be abnormally notched, lobulated, or convoluted, or it may show unusual lack of segmentation, despite other evidences of maturity. The cytoplasm may be abnormally basophilic. The granules may be abnormally sparse, they may stain abnormally, or different granules in the same cell may stain differently. Such cells are often seen in leucemia, but they are not uncommon in infections and other conditions in which there is a sharp stimulus to new cell formation. Pathological lymphocytes are especially common in such infections. It is important to remember that they do not usually indicate leucemia, and that, as a rule, they do not have a bad prognostic significance.

More significant are the changes in the leucocytes which indicate toxic injury. One of the commonest evidences of this is vacuolization of the cytoplasm, or occasionally of the nucleus. The nucleus may show abnormalities of staining. There may be a tendency for it to become swollen and poor in chromatin, and to lose its finer structural markings, or it may take a uniform dense pycnotic stain. There may be a partial or complete loss of granules, or the granules may stain poorly or show abnormality in size or tint. Such changes are common in severe infections, and as emphasized by Naegeli, they have a bad prognostic significance.

MISCELLANEOUS CONSIDERATIONS

Smudged Cells in many cases are due simply to poor technique, or to too thin films. However, even in well-made films there is a tendency for the larger cells to be smudged. These are apt to be especially numerous in certain cases of leucemia where there are many large fragile cells present. Such smudges have been described as basket cells. In such cases careful study should be made of fresh preparations in which these cells can be kept intact.

Even in well-made films of normal blood, however, there are almost invariably some smudges, and in some preparations these may be quite numerous. Many of them can usually be identified as polymorphonuclear neutrophiles despite the smudging. Sabin and her associates[3] have shown that in such cases fresh preparations simultaneously made and supravitally stained by her special technique show correspondingly large numbers of leucocytes which are nonmotile, and which show other evidences of degeneration. She believes that these smudges (good technique assumed) represent disintegrating cells. They vary greatly in number in the blood of the same normal individual at different hours on the same day.

While Wilson's (or Wright's) stain is highly satisfactory for the study of the mature leucocytes, it is not as satisfactory for the study of blood in

[3] Sabin, F. R., *et al.*, *Bulletin Johns Hopkins Hosp.*, 1925, xxxvii, 14.

leucemia and other conditions in which immature cells are present. The chief difficulties are these:

(1) The differentiation of myeloblasts from lymphoblasts. Unfortunately this is difficult with any method of staining, and often requires the experience of an expert. The main points of differentiation have been given in detail. One trained in the method may get additional help from Sabin's method of supravital staining with Janus green,[4] as there is some difference in the number and distribution of mitachondria in the two types of cells. However, if the clinical picture be considered in conjunction with the blood, there is little probability that this difficulty will lead to any diagnostic error of practical importance. While one may confuse an acute myeloid leucemia with an acute lymphatic leucemia, the prognosis and the therapy, or lack of it, are essentially identical.

(2) The differentiation of myelocytes from monocytes. This is a matter of great practical importance, as the diagnosis of myeloid leucemia may largely depend on it. In a well-stained film there should be little difficulty in distinguishing monocytes from myelocytes with mature well-differentiated granules, but in the case of relatively immature myelocytes with undifferentiated bluish staining granules the difficulty may be a real one. In such cases one must sometimes resort to special methods of staining. Ehrlich's triple stain (not the triacid stain) is distinctly superior in this respect, as the myelocytic granules are usually sharply stained, while the monocytic granules, if

[4] Sabin, F. R., *Bulletin Johns Hopkins Hosp.*, 1923, xxxiv, 277.

stained at all, are relatively inconspicuous. Sabin's [5] method of supravital staining with neutral red is also of great assistance. The monocytes are much more actively motile. They usually contain some red staining particles which are much coarser than any to be found in the myelocytes, and some of the particles tend to assume a rosette arrangement in the concavity of the nucleus.

Peroxidase stain: In order to assist in the differentiation of myeloid cells and monocytes from lymphocytes various peroxidase stains have been suggested. One of the simplest and most satisfactory methods is that of Goodpasture (see page 289). In films stained by this method the red cells, leucocyte nuclei, and lymphocytes are stained a bright pink. The platelets are not stained. The granules of the myeloid leucocytes are stained a deep bluish or brownish black color. In the monocytes are a smaller number of similar granules. None are ever present in the lymphocytes. The staining is the result of the oxidation of the benzidine in the stain by the hydrogen peroxide in the presence of oxidizing ferments (peroxidase) which are present in the two groups of cells mentioned, but not in the lymphocytes. The practical value of the stain is lessened by the fact that, contrary to the statements originally made, myeloblasts do not give a positive reaction. Any myeloblast-like cell which shows granules with a peroxidase stain can be shown to contain granules by the use of a polychrome-methylene blue stain like Wilson's stain, and is therefore an early myelocyte. However, any cells which do

[5] Sabin, F. R., *loc. cit.*

show granules with a peroxidase stain cannot possibly be lymphocytes.

The inexperienced worker should remember the following points in the study of stained blood films. Little or no information is to be obtained from a poorly spread or poorly stained film. It is rarely possible to identify with certainty any cell which is smudged or distorted, or which is not evenly spread out, so that the minute structural details are clearly recognizable. One should, therefore, avoid the thin portions of the film, and also the thick portions, except perhaps for the study of large fragile cells which may be smudged in those portions of the film which for other purposes would be of optimum thinness. One should not expect to be able to identify with certainty every cell in an abnormal blood, even in the best of preparations. One should begin the study of a film with a careful inspection of typical examples of the normal types of leucocytes, and thus get a clear mental picture of their appearance in that particular preparation, with special reference to the depth of staining and texture of the chromatin network of the nuclei, and the tint of both the neutrophilic and monocytic granules. One is then much better able to decide as to the identity of the less typical individual cells. If unusual cells of doubtful type are present, one should examine a large number of individual cells, preferably from more than one preparation, before coming to a decision. Quite often only a relatively few individuals may show the differential diagnostic points clearly. One may also be helped greatly by considering the other

types of cells present. Thus if cells are present which resemble myeloblasts, the presence of definite myelocytes, and particularly of cells intermediate in type between myelocytes and the suspected cell, would be strong confirmatory evidence that the latter are myeloblasts. Conversely, the absence of myelocytes, and the presence of intermediate types of lymphocytes, would indicate that the cells were probably lymphoblasts.

CHAPTER IV

THE THROMBOCYTES

THE blood platelets cannot be studied advantageously in ordinary fresh preparations, as they very quickly adhere to the glass or agglomerate into clumps and disintegrate. In preparations stained with Wilson's stain they are conspicuous objects. They are roughly circular, and from 2 to 3 microns in diameter, but larger and smaller forms are common. They possess a faintly bluish stained background, over which are scattered numerous rather fine, dull, purplish red granules which resemble chromatin in their staining reaction. The platelets have a marked tendency to form clumps in the films. One may often be seen overlying a red cell, and may then have a superficial resemblance to a malarial parasite. When so placed there is a pale unstained area in the cytoplasm of the red cell surrounding the platelet.

According to the usual methods of enumeration, normal blood contains on the average about 250,000 platelets per c.mm., with a range from 200,000 to at least 300,000. The probable error in the count is considerable and only marked variations are significant. Pratt has pointed out that the actual number of platelets, determined by his somewhat more complicated method, is nearly twice this figure (average of 450,000 per c.mm.). However, the

simpler method suggested (page 289) is sufficiently accurate for practical purposes. A rough estimate of their number may be obtained from simple inspection of the film, bearing in mind that there is normally one platelet for each 15 to 20 red cells. With some experience this gives sufficient information in most instances.

The thrombocytes are not really cells at all, but are merely fragments of cell cytoplasm. They are indispensable for normal blood coagulation. Their exact function will be considered later in the discussion of this subject.

CHAPTER V

FORMATION AND DESTRUCTION OF THE BLOOD CORPUSCLES

THE RETICULO-ENDOTHELIAL SYSTEM has been shown by the extensive investigations of the past few years to be responsible directly or indirectly for both the formation and the disposal of the blood corpuscles. For this reason it is now frequently referred to as the "hemolytopoietic system." A consideration of the cells of this tissue must constitute the introduction to this chapter.

The cells of the reticulo-endothelial system are widely scattered throughout the body, in many organs and intermingled with various other tissues. These cells are not all identical with one another morphologically, but a number of different types of cells occur within the system. As yet no distinctive histological characteristic has been discovered by which these cells can be identified positively by their morphological appearance alone. The conspicuous characteristic possessed by these cells in common is their extraordinarily active power of phagocytosis. If particulate matter such as India ink or carmine particles be injected intravenously into experimental animals, the particles are speedily engulfed by these cells, and thus removed from the circulation. They can also remove from the plasma a large number of

soluble colloidal dyes, such as pyrrhol blue, and store them as small droplets. This property of phagocytosis is not strictly limited to reticulo-endothelial cells, but in situations in which cells of other types might here and there ingest a few isolated particles, the reticulo-endothelial cells will become tremendously engorged. These cells may have the shape and appearance of ordinary endothelial cells, or they may be elongated and irregular in shape (reticulum cells). Such reticulum cells are found widely scattered over the body in the connective tissue about the blood vessels. Similar phagocytic activity is shown by the fixed endothelial cells in a few special regions of the body, especially the sinuses of the lymph nodes, the blood sinuses of the spleen, the capillaries of the liver (the stellate cells of Kupfer), the bone marrow, the adrenal cortex, and the hypophysis. In addition to these fixed cells, the reticulo-endothelial system includes the monocytes of the blood, and the large wandering cells of the tissues, variously called clasmatocytes, histiocytes, endotheliocytes, splenocytes, or macrophages—all essentially synonymous terms. These wandering cells may be regarded as specialized derivatives of the fixed cells previously mentioned. According to Sabin the monocytes are derived from the reticulum cells, while the clasmatocytes are derived from the endothelium. The endothelial cells of the bulk of the capillaries of the body and the mesothelial cells lining the serous cavities do not belong to the reticulo-endothelial system.

The *functions* of this tissue are numerous and im-

portant.[1] The primitive cells of this system are believed by many to be the ultimate source from which the more highly specialized precursors of all the different types of blood cells are derived. They act as scavengers, removing from the circulation all sorts of particulate matter, cell fragments, and (often) bacteria. They may also remove from the plasma colloidal substances in solution, such as hemoglobin. They have the capacity of storing ingested material which is not digested by them, and in certain disturbances of metabolism (Gaucher's disease, Niemann's disease) there is a tremendous hyperplasia of these cells, which are loaded with cerebroside or lipoid granules. They are probably the chief source of the antibacterial substances of the serum, and they play an important part in the local defensive reaction to many acute and chronic infections, especially tuberculosis, forming the epithelioid cells and giant cells of the tubercle.

Origin of the Erythrocytes. The formation of red cells begins at an early stage in the development of the embryo, and they are the first of the blood cells to be differentiated. They are formed at first within the vessels, from groups of cells, "blood islands," which are derived from the endothelium lining the vessels. During this early stage of development the primitive erythroblasts are megaloblastic in type. Later in fetal development it is generally believed that in mammals red-cell formation becomes extravascular, and with this change

[1] Sacks, B., "The reticulo-endothelial system." *Physiol. Reviews*, 1926, vi, 504.

the megaloblasts gradually disappear and are replaced by normoblasts. In adult life, in response to a sufficiently profound stimulus, there may be, in some individuals at least, a reversion to the fetal megaloblastic type of cell.

This erythropoietic (red blood cell-forming) tissue in the earliest stages of development is pretty generally distributed throughout the embryo, but soon largely disappears except in the liver, in which it proliferates extensively. Later the spleen participates in the process, and still later the bone marrow. At birth this tissue has largely disappeared from its earlier sites, and is mainly confined to the bone marrow, practically all the bones participating. Later still it disappears from the long bones, so that in the normal adult it is confined to the short bones, especially the ribs and vertebræ. This disappearance from the other organs is perhaps not absolutely complete. In response to a sufficiently powerful stimulus there may be a reappearance of erythroblastic tissue in these abandoned areas. Some observers believe that this is due to a hyperplasia of minute islands of erythroblastic tissue which have remained dormant in these locations since fetal life. Others believe that the tissue springs *de novo* from primitive reticulo-endothelial cells which are known to be present in these locations. This distinction seems relatively unimportant, but it has given rise to much acrimonious debate. In the adult this hyperplasia first appears in the long bones, such as the femur, and not rarely also in the spleen. Rarely it may appear in the liver, and even in other parts

of the body. The more profound the stimulus, the more extensive is the proliferation and the greater is the tendency of the tissue to revert to the primitive megaloblastic type of cell.

Origin of the Granular Leucocytes. These cells also appear quite early in embryonic life. The primitive (reticulum) cells appear in the perivascular spaces around the vessels, and are of the myeloblastic type. These foci at first are widely distributed, but soon disappear largely except in the liver. Later they appear in the spleen, and finally in the bone marrow, which is practically the sole site of the leucoblastic tissue in the normal adult. As development proceeds, the myeloblasts are gradually replaced by myelocytes. The latter cells are constantly undergoing active cell division, producing more of their own kind, while from some of them the granular leucocytes of the blood develop by a process of maturation.

There may be a reappearance and hyperplasia of this myeloid tissue also, in these various areas, if an adequate stimulus arises. This may appear not only in the marrow of the long bones, the spleen, and liver, but in a great variety of other organs, including the kidneys, the skin, and the lymph glands, from which the lymphocytes may largely be crowded out. This is not due to a transformation of lymphocytes into myelocytes. It is uncertain whether the hyperplastic tissue springs from preëxisting minute foci of myelocytes or from primitive reticulum cells.

Origin of the Monocytes. There is still dispute

as to the origin of these cells. Sabin[2] has brought forward strong evidence that they arise from reticulum cells, probably in part in the marrow, but in other organs as well. Naegeli claims that they arise in the bone marrow from a myeloblast-like precursor. It is quite certain that they are not of lymphatic origin, and that they are just as distinct from the lymphocytes as are the granular leucocytes, in origin, in structure, and in function.

Origin of the Lymphocytes. These are the last of the main types of blood cells to appear during the development of the embryo. They are formed in the lymph follicles, wherever these may be located: in the lymph glands, the spleen (Malpighian follicles), in the walls of the digestive tract, the tonsils and pharynx; and in minute foci of lymphoid tissue which are widely disseminated in many organs, including the skin, the walls of the bronchi, the connective tissue framework of the liver, the salivary and lacrymal glands, and in the adventitia of the blood vessels of the bone marrow. On occasion the lymphoid tissue in any or almost all of these situations may proliferate, and even form tumor-like masses. The immediate precursors of the circulating lymphocytes are the large cells at the center of the follicles, many of which may be seen in active division.

Origin of the Thrombocytes. The work of Wright has proved clearly that the platelets are formed in the bone marrow, from the giant cells

[2] Sabin, F. R., "On the origin of the cells of the blood." *Physiol. Reviews*, 1922, ii, 58.

(megakaryocytes), by the pinching off of portions of their long cytoplasmic processes.

The question naturally arises, is there not some *primitive "stem cell"* in the body which may give rise to all these types of white cells, and perhaps to the red cells also? This question has been actively disputed by hematologists for many years and is not yet settled. Until recently the majority of workers have favored this "monophyletic" theory. One of the strongest points supporting this view is the difficulty or impossibility of distinguishing between the immature forms of these different types of cells in fixed and stained preparations. However, studies by Sabin and others, using vital staining methods, tend to show that there are specific differences between these various types of cells from a very early stage. In brief, it is probable that under normal conditions the existing precursors of each of these main cell types are definitely differentiated at birth, each giving rise only to its own specialized mature cell form. There is no convincing evidence that a "metaplasia" of one differentiated type of blood cell into another ever occurs. Embryologically these cells must have a common ancestor, and it is fairly clear that this is to be sought among the primitive cells of the reticuloendothelial system. Whether such a primitive cell is present in the adult is uncertain. Sabin believes that cells are present in the adult which can give rise to any one of the three main types of leucocytes. One must go farther back, probably, to find an ancestor common to both leucocytes and red cells.

The Mechanism Which Normally Prevents the Entrance of Immature Cells Into the Circulation is a problem which has aroused a great deal of interest and speculation. The most plausible hypothesis explains this mechanically, and is based on present conceptions of the histological structure of the marrow. The active marrow consists of a "parenchyma" of the special marrow cells (immature blood cells and their precursors), with some fat cells, supported by a fine connective tissue framework. This is permeated by many vascular spaces or sinuses three times or more the diameter of ordinary capillaries, through which the blood circulates sluggishly. It is now known that these spaces are lined throughout by a thin layer of endothelial cells with their cytoplasmic processes, which may be thought of as forming a barrier to the entrance of the cells of the marrow parenchyma into the circulation. Through this barrier, it is claimed, the mature nonnucleated elastic red cells can be squeezed by pressure from the growing tissues, while the nucleated red cells are held back by their lesser elasticity and greater bulk. The mature leucocytes can pass through the barrier by reason of their ameboid activity, while the immature cells, which are nonmotile, are held back. Injury to the endothelial lining of the vascular spaces, or a markedly increased pressure against it, due to unrestricted multiplication of the marrow cells, may result in such breaks in continuity that normoblasts and myelocytes can also pass through, together with such other immature cells as are not too large to pass through the capillary spaces.

Summary. Except perhaps for the monocytes, the cells of normal blood are formed mainly in two tissues, the lymphoid tissue and the red marrow of the short bones. In the latter we find side by side, and inextricably intermingled, the production of red cells, granular leucocytes, and platelets. As might be expected, any process or condition which markedly influences the production of any one of these three groups of cells is likely to cause a disturbance of that of the other two, which is qualitatively similar, although it may differ in degree. Thus the stimulation of the erythropoietic tissue of the marrow that follows hemorrhage is associated as a rule with an increase in the granular leucocytes and in the platelets. On the other hand, the depressing effect on the leucopoietic tissue of radiation or of benzol poisoning is accompanied by a definite reduction in the formation of platelets, and to a less extent of red cells also. The lymphoid tissue is relatively independent of the marrow, but any influence which profoundly stimulates or depresses the marrow may exert an appreciable influence on the formation of lymphocytes as well. An appreciation of the close relationship existing between these different hemopoietic tissues is necessary for a clear understanding of the blood changes which occur in disease.

Method of Removal of the Erythrocytes. The average life of the red blood cells under normal conditions is probably about four weeks, although some estimates place it at less than half this figure. This means that there is removed from

the circulation daily a mass of red cells corresponding to between 125 cc. and 250 cc. of blood. The exact details of this process are not yet thoroughly understood. It is certain that the cells do not normally undergo lysis in the circulation. To a limited extent circulating red cells may be engulfed by macrophages and thus removed from the circulation throughout the reticulo-endothelial system. This process is quite marked in certain diseases, such as pernicious anemia, but in the normal individual it seems to play a minor rôle. Rous and Robertson[3] have furnished evidence that as a result of the buffetings that the cells encounter in the circulation they are eventually broken up, but without liberation of hemoglobin into the plasma. These cell fragments (the larger ones are the microcytes of pathological blood) are filtered out largely (probably) in the spleen, where they are engulfed by macrophages and the process of disintegration is completed. The reticulo-endothelial cells of the liver, the lymph glands, and possibly the bone marrow doubtless participate in this, and after splenectomy these organs can take over the entire process.

The hemoglobin of the disintegrated cells is split up, and the iron it contains is avidly retained in the body, being stored largely in the spleen and liver until it is again worked up into new hemoglobin. The balance of the hemoglobin molecule, or a portion of it, is converted into bilirubin, probably by the reticulo-endothelial cells themselves. It is then

[3] Rous, P., "Destruction of the red blood cells." *Physiol. Reviews*, 1923, iii, 75.

carried by the plasma to the liver, where it is taken up by the glandular cells, and excreted in the bile. According to Whipple, the bile pigments thus excreted represent purely waste products, and are not utilized in the formation of new hemoglobin. However, this point is still in dispute.

Removal of the spleen, by diminishing the volume of the reticulo-endothelial tissue, might be expected to lessen the rate of blood destruction. This it probably does to some extent, even in the normal individual, since the resistance of the red cells to hemolysis is increased. However, the capacity of the normal body to dispose of red cells is not seriously lessened by splenectomy. Following the operation there is often evidence of compensatory hyperplasia of the reticulo-endothelial tissue elsewhere. Pearce and Krumbhaar[4] do not regard the spleen as of primary importance in the process of blood destruction in the normal individual, but admit its great significance in certain diseases associated with increased blood destruction. They found that experimental splenectomy in normal animals was followed by a transient anemia which they attribute to the loss of a stimulating action exerted by the spleen on the marrow. On the other hand, in some diseases in man, associated with increased blood cell destruction, such as familial hemolytic jaundice and thrombopenic purpura, splenectomy is followed not only by a great decrease in the rate of blood cell destruction, but by such marked evidences of

[4] Krumbhaar, E. B., "Functions of the spleen." *Physiol. Reviews*, 1926, vi. 160.

increased blood cell formation that one seems forced to the conclusion that the bone marrow has been released from some inhibiting influence that had previously been exerted by the spleen. Despite the subsequent hyperplasia of reticulo-endothelial tissue in other organs, this stimulating effect on the marrow is often lasting.

The Method of Removal of the Blood Platelets is not definitely known, but the spleen is suspected of playing the chief rôle. Their average life is short, probably about three or four days.

The Method of Removal of the Leucocytes is also incompletely worked out. The life of the individual cell, or at least its stay in the blood stream, is probably very short. It has been estimated for the neutrophiles at three days. The demonstration by Sabin of showers of dying and disintegrating leucocytes in the circulation has already been referred to. Such cellular detritus is probably removed by the large phagocytic cells of the reticulo-endothelial system. The life of the lymphocytes seems to be even briefer, probably less than one day, since Rous and others have shown that the number of lymphocytes entering the circulation during one day is greater than the number present in the blood at one given time. Disintegrating lymphocytes do not appear in large numbers in the circulation as do the neutrophiles. While it is not improbable that some are removed by phagocytosis, Bunting and Huston have shown that large numbers of them leave the body by migrating onto the surface of the mucous membranes, especially of the

gastrointestinal tract. The chief functions of the white cells are probably not fulfilled while they are in the circulation, and they may be regarded in the main as transients, utilizing the blood stream as a means of transportation.

CHAPTER VI

LEUCOCYTOSIS AND LEUCOPENIA

ALTERATIONS in the number of white blood cells occur in a great variety of conditions in which the blood changes are purely secondary and symptomatic. There may be an increase or a decrease in the total number of cells per c.mm. or an alteration in the relative number of the different types of cells without a significant change in the total count. An increase in the total count above 10,000 per c.mm. is designated as a *leucocytosis*. If the increase is made up mainly of polymorphonuclear neutrophiles, it constitutes a *neutrophilic leucocytosis*, and as this is the case in the great majority of instances it is often called simply a leucocytosis. Such an increase is known as an absolute neutrophilic leucocytosis, as distinguished from a relative neutrophilic leucocytosis, a condition in which the percentage of these cells is increased but in which there is no rise in the total count. The diagnostic significance of a relative neutrophilic leucocytosis is substantially the same as that of an absolute leucocytosis of slight degree.

An eosinophilic leucocytosis is usually spoken of as an *eosinophilia*, and an increase in lymphocytes as a *lymphocytosis*. The term *monocytosis* (or mononucleosis) may be used in case of an increase

in the cells of this group. In each case the increase may be absolute, or relative only.

A reduction in the total white cell count below 5,000 per c.mm. constitutes a *leucopenia.* This is largely at the expense of the neutrophiles.

Rapid and fairly marked changes in the total white cell count may occur in the normal individual as a result of a variety of stimuli, quite apart from any disease process. These fluctuations may be so rapid that new cell formation is quite out of the question, and they must be due to changes in the distribution of the cells, a migration from the visceral and marrow capillaries into the peripheral circulation, and vice versa. Such changes may follow a cold bath, severe muscular exertion, or possibly a heavy meal, and in some cases no adequate explanation may be found. Such increases are sometimes spoken of as physiological leucocytoses, in contrast with those which are associated with infection or other disease processes.

Sabin and her associates [1] have called attention to the unexpectedly marked rhythmical fluctuations in the total white cell count which may occur in normal individuals when hourly counts are made throughout the day, even when the individual is fasting. These counts may range from 5,000 to 10,000 per c.mm., despite the absence of any adequate external stimulus to account for the variation. These authors emphasize the necessity of caution in interpreting the significance of corresponding changes in the count in pathological conditions. Their observa-

[1] Sabin, F. R., *et al.*, *Bulletin Johns Hopkins Hosp.*, 1925, xxxvii, 14.

tions also raise grave doubts as to the occurrence of a digestive leucocytosis, since just as marked variations in the count occurred in fasting individuals.

A Neutrophilic Leucocytosis occurs in the following conditions:

(1) Infections:

A neutrophilic leucocytosis occurs in most acute infections with the exception of those listed below as causing a leucopenia or an increase in lymphocytes. It is especially marked and constant in infections with the pyogenic cocci, in pneumonia, meningitis, acute rheumatic fever, acute inflammatory conditions within the abdomen, scarlet fever, and simple acute tonsillitis. It is unusual in uncomplicated tuberculosis. Its absence is an important diagnostic point in typhoid fever, malaria, measles, and influenza.

The degree of rise varies greatly, but the count is usually between 12,000 and 20,000. Counts of 30,000 are not very unusual and they occasionally rise to 50,000. Rarely indeed one sees counts as high as 100,000. The possibility of the occurrence of such high counts in infections must be kept in mind in considering the diagnosis of leucemia.

(2) Intoxications:

A neutrophilic leucocytosis may be met with in uremia, in illuminating gas poisoning, in lead poisoning, in gout during an acute attack, and after the administration of certain drugs, such as turpentine, acetanilid and allied drugs, potassium chlorate, digitalis, and salvarsan. In other cases these drugs may cause a leucopenia. It is important to remember

that a leucocytosis of 20,000 may be met with in lead colic.

(3) Following acute hemorrhage.

(4) Pregnancy:

During the last few months of pregnancy and during the puerpurium there is normally a rise in leucocytes. This rise is slight during the pregnancy, may be considerable during labor and the day or two following, after which it gradually falls again to normal. This must be regarded as physiological, but it materially lessens the significance of the white cell count so far as the recognition of complicating infections is concerned.

(5) Postoperative leucocytoses:

For a short period after operation (12 to possibly 36 hours) there may be a moderate rise in leucocytes which is not demonstrably due to infection. It is more apt to appear where extensive tissue injury has occurred.

(6) Malignant growths:

It is seen most frequently in case of growths involving the liver, extensive growths of the gastro-intestinal tract, and metastases to the bone marrow.

(7) Acute and chronic myeloid leucemia.

Leucocytic Index (Arneth). Elaborate studies of the minor changes in the leucocytes in disease have been made, notably by Arneth and his pupils. Arneth studied particularly the different types of neutrophilic leucocytes present. These he subdivided according to his conception of the stage of development (maturity) of the cells into five main classes. In the first class ("left-hand" group) he put the myelocytes and metamyelocytes with in-

dented or band-shaped nuclei. The polymorphonuclear neutrophiles he subdivided into four additional classes, according to the number of lobes of the nucleus. Cells in Class 2 have 2 lobes, while cells in Class 5, the "right-hand" group, have 5 or more lobes. He regarded the age (maturity) of the cell as directly proportional to the degree of lobulation of the nucleus. The cells in the left-hand groups would, therefore, be less mature than those in the right-hand groups. If the proportion of neutrophilic cells in the first classes is increased above the average normal figures (at the expense of the right-hand groups) the "index" (of Arneth) is said to show a "*shift to the left.*" The extent of this shift would measure approximately the degree of immaturity of the leucocytes. Such a shift is to be expected in some degree as a response to almost any type of infection. In milder cases the change is slight, but in severe or progressing cases it is marked. In general, according to Arneth, the greater the shift, the more severe is the infection, the less adequate is the response of the marrow, and the graver is the prognosis. The shift in the index he regarded as far more significant than the height of the total leucocyte count.

These views of Arneth have not secured general recognition. There is no doubt that the cells included in his Class 1 (or at least many of them) are really immature cells. However, it has been pretty definitely shown that the degree of maturity of the cells with segmented nuclei is not directly proportional to the number of segments in the nu-

cleus. Moreover, in severe infections toxic changes in the leucocytes make it difficult to count the number of segments accurately. It has, therefore, been necessary to abandon Arneth's elaborate classification.

Other workers, recognizing that there was some truth in Arneth's ideas, have attempted to restate his theory in a simplified and more defensible form. Thus Schilling divides the neutrophilic leucocytes into immature forms, equivalent to Arneth's Class 1, and mature forms (Arneth's Classes 2 to 5 inclusive). The immature forms he also subdivided into (1) myelocytes with round or oval nuclei (absent in normal blood); (2) young metamyelocytes with indented nuclei (almost none in normal blood); and (3) old metamyelocytes with band-shaped nuclei (4.0 per cent in normal blood). The mature forms with segmented nuclei constitute 63 per cent of the total normal count. An increase in the percentage of the immature forms constitutes a shift to the left in the index; it can be demonstrated without much technical difficulty when it exists; and according to Schilling it has the same significance as was attributed by Arneth to a change in his index.[2] Naegeli criticized this classification also, because, particularly in infections, some cells classified as immature on the basis of the shape of their nuclei are really pathological leucocytes with nuclei which in their structural details show evidence of maturity.

[2] Reviewed by Piney, A., in *Recent Advances in Hematology*, Ed. 2, 1928.

Significance of a Leucocytosis. In general a leucocytosis may be regarded as the effective response of the bone marrow to an increased functional demand for leucocytes, such as may arise during an infection. The qualitative and quantitative character of the response will depend on two factors: on the intensity of the stimulus (the severity of the infection) and on the reacting power of the individual. The latter will depend in part on the inherent quality of the marrow of the individual, and in part on the degree to which the marrow has been directly injured and depressed by the infection. A very mild infection may call forth an insignificant response. On the other hand, if the intoxication is overwhelming, or if the individual is very debilitated and incapable of reacting effectively, a leucocytosis may fail to appear, or there may even be a reduction in the total leucocyte count, and particularly in the neutrophiles. If the infected individual possesses adequate reacting power, the degree of leucocytosis is very roughly proportional to the severity of the infection.

In case of a moderately severe infection, with good reacting power of the marrow, one may expect a marked increase in the total leucocyte count (*e.g.*, to 20,000), due entirely to an increase in the neutrophiles, and the percentage of these cells will rise to, perhaps, 90 per cent. If these be examined in detail there will usually be an increase in the metamyelocytes (a shift to the left in the index). The eosinophiles and basophiles may be scanty or absent. The total number of the lymphocytes will be somewhat diminished, as well as the relative number.

In more severe cases the following points tend to have a bad prognostic significance:

(1) An extremely high total leucocyte count, with a very high percentage of neutrophiles.

(2) The failure to develop a leucocytosis, or an actual leucopenia.

(3) The presence of a considerable percentage of immature cells, especially if they outnumber the mature cells (Schilling). When many myelocytes are present the condition is sometimes referred to as a leucemoid reaction. Such cases may simulate acute leucemia.

(4) Absence of eosinophiles.

(5) A marked reduction in the absolute number of lymphocytes.

(6) The presence of many cells showing toxic-degenerative changes in the nucleus or cytoplasm. Naegeli especially emphasizes the bad significance of the last two points.

Conversely, favorable signs are a falling total leucocyte count together with a falling percentage of neutrophiles and a reduction in the proportion of immature forms, a reappearance of eosinophiles, an increase in the total number of lymphocytes, and the absence or diminution in number of leucocytes showing toxic changes.

It must be kept in mind that these are general tendencies only, to which there are many individual exceptions, and at best the blood changes show only one phase of the situation. Not too much stress should be placed on the result of a single examination, but where in a series of successive examinations there is a progressive increase in those changes in

the blood mentioned as having an unfavorable significance, a fatal outcome is highly probable unless some effective radical measures of treatment can be instituted.

LEUCOPENIA

While all types of cells may be proportionately reduced, any notable reduction in the count below 5,000 cells per c.mm. is mainly at the expense of the neutrophiles. It may be met with in the following conditions:

(1) Cachectic and debilitated states.

(2) Infections: regularly in typhoid fever, measles, influenza, and malaria; and occasionally in extremely severe infections, especially if associated with general sepsis, in which a leucocytosis is the rule.

(3) Agranulocytic angina.

(4) Intoxications: such as chronic poisoning with benzol, acetanilid, and other blood poisons, rarely after salvarsan injections, or other arsenicals, lead, mercury, etc.

(5) After excessive exposure to radium or X-ray.

(6) Exhaustion of the marrow, as seen in pernicious anemia, in rare cases of acute leucemia, and in the terminal stage of chronic leucemia. A similar result is produced by the extensive invasion of the bone marrow by other tissues, particularly by malignant neoplasms. The lymphoid tissue may similarly be crowded out of the lymph glands, as in some cases of Hodgkin's disease, tuberculosis, and malignant tumors.

EOSINOPHILIA

As an arbitrary upper limit of normal an absolute count of 250 cells per c.mm. or about 3 per cent of the average normal count, is usually taken. Eosinophilia may occur in a widely diversified lot of pathological conditions. Many of these can be classified roughly in three groups, which, however, may not be mutually exclusive.

(1) Infection with animal parasites.

Eosinophilia may be present in almost any form of parasitic infection. Trichiniasis is the most striking example. Here the phenomenon is almost constant, and the degree of rise may be enormous: 10 to 70 per cent of a total count which may be as high as 35,000. The neutrophiles may be absolutely as well as relatively diminished. With intestinal parasites, such as hookworm, tenia saginata, ascaris, strongyloides, hydatid cyst, it is much less constant in its occurrence, but at times may be considerable in degree, from 5 to 35 per cent. In severe hookworm infection some increase in eosinophiles is usually present. In amebic dysentery it is the exception to have any notable increase.

(2) Skin diseases.

The degree of eosinophilia in these conditions seems to depend on the extent of skin involvement, rather than on the type of disturbance present. Chemical irritation of the skin can cause it. The rise is usually moderate but may be high.

(3) Reactions to foreign protein in hypersensitive individuals:

Here may be included the eosinophilia of bron-

chial asthma, of serum disease, of urticaria and angioneurotic edema, and probably that occasionally seen in Henoch's purpura, and after tuberculin reactions.

There remains a heterogeneous group of conditions in which an eosinophilia occasionally occurs. Infections associated with a reduction of the neutrophiles show as a rule a similar reduction in the eosinophiles. Those with a neutrophilic leucocytosis during the acute stages of the infection usually show an absolute as well as a relative decrease in the eosinophiles. With or just preceding the advent of recovery there is apt to be a return of these cells to normal, or above, which in general has a favorable prognostic significance. Scarlet fever is an exception, as there is usually a slight eosinophilia from the start, a fact which sometimes has diagnostic significance.

Hodgkin's disease is not regularly accompanied by an eosinophilia, but counts as high as 60 per cent have been observed. Splenectomy is usually followed by an eosinophilia of moderate degree which may persist as long as a year. An eosinophilia occurs occasionally associated with certain tumors of the ovary, in focal lesions of the bone marrow, and regularly in chronic myeloid leucemia. It has been reported as an exceptional occurrence in a great variety of other conditions, for a description of which the reader is referred to the larger texts.

A Basophilic Leucocytosis occurs in most cases of chronic myeloid leucemia, but is infrequent in other conditions, and is of no known diagnostic value.

LYMPHOCYTOSIS

A relative lymphocytosis is present in practically all the conditions listed above as showing a reduction in neutrophilic leucocytes. An absolute lymphocytosis is met with in the following conditions:

(1) Normal infants after the first week. This averages roughly about 60 per cent, or a total of 4,000 to 5,000 per c.mm. This lymphocytosis persists in varying degree during the first few years of childhood, gradually diminishing until the usual adult blood picture is reached. There is considerable variation in the age at which this change takes place. In some cases the adult formula is found in comparatively young infants, in others the lymphocytosis persists until the eighth or tenth year. In young children there may be an increase in the lymphocytes in conditions which in older persons would usually cause a neutrophilic leucocytosis. This is also an inconstant and variable phenomenon.

(2) Infections:

In *whooping cough* during the early stages of the disease there is usually a well-marked rise in the total leucocyte count, due largely to an increase in small lymphocytes. In average cases there may be 20,000 to 40,000, with 60 to 80 per cent of lymphocytes. Counts of over 100,000 are on record. The blood in such cases might easily be mistaken for that of lymphatic leucemia. Not every case shows a well-defined lymphocytosis, and in the presence of secondary infections there may be a neutrophilic leucocytosis, although with them also a lymphocytosis is the rule.

Infectious mononucleosis (so-called), or glandular fever, is an acute infection of unknown cause occurring sporadically and in epidemics, chiefly in children and young adults, characterized as a rule by an acute onset with fever, sore throat, signs of acute tonsillitis, marked swelling of the cervical lymph glands, and often enlargement of the spleen. In some cases there is well-marked general lymph glandular enlargement, and the cervical glands may be huge, suggesting (in conjunction with the blood picture) an acute lymphatic leucemia. The disease runs a benign course, with fever lasting one to three weeks. The lymphocytosis is usually evident from the start, but may reach its maximum only after 2 to 3 weeks. There may be a total count of 12,000 to 20,000 cells, of which as many as 85 per cent may be lymphocytes. The striking feature of the blood picture is the large number and great variety of pathological lymphocytes. Azure granules are usually abundant. Many cells show deeply basophilic cytoplasm, like plasma cells. The monocytes are not increased and the term mononucleosis is unfortunate because misleading. Until definite information as to the etiology is obtained, "benign infectious lymphocytosis" would be a far better descriptive term. There is no anemia, no reduction in platelets, and no tendency to bleed. In some cases the diagnostic difficulties are increased by the persistence of the glandular enlargement and the lymphocytosis for many weeks or months.

Cabot questions the propriety of considering this condition as a clinical entity, and regards it rather as an unusual type of blood reaction, due to a peculi-

arity of the individual. However, some cases have been observed which subsequently reacted to an acute infection of some other type in the usual manner, with a neutrophilic leucocytosis. While no proof has yet been furnished that the condition has a specific etiology, the clinical picture is so characteristic and well-defined that the assumption that we are dealing with a disease entity seems probable, even though not definitely proved.

In *German measles* the total count may be normal or moderately increased. The striking feature is the marked increase in lymphocytes appearing about the third day. There are numerous immature and pathological forms, and (especially characteristic) numerous plasma cells, which may vary from 1 to 10 per cent, or in extreme cases 30 per cent. Such an increase is practically unknown in other conditions except in rare cases of lymphatic leucemia. The lymphocytosis may be lacking in cases with little or no glandular enlargement. A few plasma cells may be found in any infection associated with lymphocytosis.

A moderate lymphocytosis is often seen in *Malta fever*, in *mumps* (except with orchitis), and in some cases of *tuberculosis*, chiefly uncomplicated cases of moderate severity which are running a favorable course.

(3) Convalescence from infections:

During the acute stage of most infections associated with a neutrophilic leucocytosis there is an absolute as well as a relative diminution of the lymphocytes. This may also be seen in typhoid fever

and tuberculosis. With beginning recovery there is an increase in the absolute number of the lymphocytes from their low figure, and during convalescence this often amounts to an absolute and relative increase above the normal figures. In occasional cases this may be considerable and, if unexpectedly encountered, it may be confusing. This fact is not generally appreciated, because interest in the minute details of the blood is apt to flag with the recovery of the patient.

(4) Intoxications of various sorts:

In these conditions there is usually some depression of the neutrophiles, with a relative lymphocytosis. Exophthalmic goiter frequently shows such a relative lymphocytosis. In some of the severer cases there may be a definite absolute lymphocytosis, which by some is regarded as having a bad prognostic significance. A relative lymphocytosis is also found in some cases of myxedema.

(5) Lymphatic leucemia.

MONOCYTOSIS, OR MONONUCLEOSIS

A notable increase in these cells is comparatively infrequent, and does not occur with sufficient constancy in any condition to be of much practical diagnostic value. An absolute increase occurs occasionally in typhoid fever, subacute bacterial endocarditis, typhus, relapsing fever, Hodgkin's disease, smallpox, malaria, and in salvarsan reactions. Infections causing a neutrophilic leucocytosis may show some absolute increase in monocytes, although the percentage is usually low.

MYELOCYTOSIS

This term is sometimes applied to blood containing appreciable numbers of myelocytes. An isolated myelocyte may occasionally be found in normal blood (Sabin).[3] In films stained by Wilson's stain such a cell is apt to be mistaken for a monocyte. Myelocytosis may occur in:

(1) Myeloid leucemia, acute and chronic. The presence of these cells in considerable numbers is the constant and characteristic feature of this disease. Even in remissions with a total leucocyte count which is within normal limits, an appreciable number of these cells persist.

(2) Neutrophilic leucocytoses of marked degree. Regardless of the condition causing the leucocytosis, a few myelocytes are often present. This is particularly apt to be the case in children. In exceptional cases the number may be high, and may strongly suggest leucemia (pneumonia, scarlet fever, sepsis). Such a phenomenon has been termed a leucemoid reaction.[4]

(3) Severe infections with functional injury to the bone marrow, without a leucocytosis or even with leucopenia. The presence of myelocytes is a bad prognostic sign. Such cases show a maximal degree of the shift to the left of Arneth's index.

(4) Intoxications of various sorts similarly injuring the marrow.

[3] Sabin, F. R., *et al.*, *Bulletin Johns Hopkins Hosp.*, 1925, xxxvii, 14.

[4] Krumbhaar, E. B., *Am. Jour. Med. Sci.*, 1926, clxxii, 519.

(5) Pernicious anemia, and other types of severe anemia. The so-called anemia pseudoleucemica infantum is an extreme example. In adults with pernicious anemia myelocytes may rarely be present in such numbers as to offer diagnostic difficulties.

(6) Focal diseases of the bone marrow, such as metastatic neoplasms.

CHAPTER VII

CLASSIFICATION OF THE ANEMIAS

Definition. By anemia is meant a reduction below the normal in the amount of hemoglobin (and usually also in the number of red corpuscles) per unit volume of blood. Anemia is not a disease of the blood, but a state of the blood which is a symptom of a great variety of diseases. In a majority of the cases anemia is the result of injury to the erythropoietic tissue of the marrow, rather than to the cells that are present in the circulating blood. It is an indication of marrow injury or insufficiency. By a study of the blood one can gauge roughly the degree of injury to the marrow, and in some cases can draw some conclusions as to the character of the disease process which has caused the injury.

Numerous attempts have been made to classify the anemias, but none is altogether satisfactory. The most logical classification is according to the pathogenesis. Thus one may distinguish anemias due to:

(1) Loss of blood; that is, external hemorrhage, including hemorrhage into the lumen of the gastrointestinal tract, the respiratory and genito-urinary passages: Posthemorrhagic Anemias.

(2) Destruction of blood within the body, Hemolytic Anemias.

(3) Inadequate blood formation, Aplastic Anemias.

The posthemorrhagic anemias make up a fairly well differentiated group, but the others cannot be apportioned satisfactorily between groups 2 and 3 for the reason that in the majority of cases the two conditions of increased blood destruction and inadequate formation are inextricably intermingled. In only a few conditions can one process or the other be recognized as clearly dominating the picture. However, this classification gives an interesting and instructive point of view from which to consider the anemias. It is not possible to differentiate with certainty between these three classes of anemias by a study of the blood changes alone. However, well-marked typical examples of each class show fairly definite characteristics which will be discussed later.

The classification most widely used is the old one which subdivides the anemias according to their etiology into:

(1) Secondary Anemias, those due to some ascertained cause, such as hemorrhage, cancer, or an infection.

(2) Primary Anemias, those without any ascertained cause. Because no obvious external cause was found it was (erroneously) assumed that the disease originates in and primarily involves the blood. This is the primary pernicious anemia of Addison.

This classification is quite illogical because it is based primarily on ignorance, and also because it makes no attempt to separate different types of secondary anemia which are entirely unrelated to each other. However, it has served a useful purpose

in emphasizing the differences between pernicious anemia and other types of anemia.

The morphological changes in the blood in primary pernicious anemia (high color index, large dark cells) are quite distinctive and different from those seen in most secondary anemias (low color index, small pale cells), so that it is usually possible to distinguish these two groups from one another by the blood changes alone. As a result the terms, primary anemia and secondary anemia, have frequently been applied to the morphological changes in the blood characteristic of these two groups of anemias. This is unsatisfactory also, because blood changes of the primary anemia type occur in several conditions the cause of which is definitely established (*e.g.*, fish tapeworm infestation); and because there are anemias which show the secondary anemia type of blood change, the cause of which is unknown (*e.g.*, chlorosis). If a classification is to be made on morphological grounds, it would be more logical and precise to use morphological terms to designate the groups: *megalocytic anemia* for the so-called primary anemia blood changes, and *microcytic anemia* for the "secondary" anemias (as has been suggested by Hampson and Shackle).[1]

[1] Guy's Hospital Reports, 1924, lxxiv, 193.

CHAPTER VIII

POSTHEMORRHAGIC ANEMIA

ACUTE POSTHEMORRHAGIC ANEMIA

AFTER a single severe hemorrhage in an otherwise healthy individual, unless quickly fatal, the following changes in the blood will occur.

A blood count made very soon after the hemorrhage has occurred will show a normal red cell count and normal hemoglobin figures. The only immediate result is a diminution of blood volume. The immediate danger from acute hemorrhage is from the effect of the loss of blood volume (fluid) upon the circulatory system, and not from lack of red cells or hemoglobin. The first reaction on the part of the body to hemorrhage is the passage of fluid from the tissues into the vessels to restore the lost volume. As this proceeds the remaining red cells are distributed through an increasing volume of blood, and, therefore, the number of red cells and the amount of hemoglobin per c.mm. will fall progressively. Each individual cell will be quite normal, and the color index will be normal. This process of dilution begins at once but is gradual and requires some hours for its completion, so that the minimum figure for hemoglobin may be reached only 12 hours or even more after bleeding has ceased. Successive hemoglobin estimations, there-

fore, will eventually give a good indication as to the total amount of blood lost, but not as to the persistence of bleeding during the few hours (6 to 12) preceding the examination.

The lack of red cells and hemoglobin acts as a stimulant to the erythropoietic tissue of the marrow, and to some extent to the entire hemopoietic tissue. As a result there is first of all a rapid outpouring of red cells from the spleen, marrow, etc. Those first appearing are fully mature normal red cells which may be looked upon as a surplus stock which has been stored in these organs to supply the ordinary physiological demands. This surplus is quickly exhausted, and further need must be met by an accelerated production of new cells. That this actually occurs is clearly shown by the pathological appearance of the marrow. There is marked hyperplasia of the red marrow, with increase in its bulk. The cells show signs of active proliferation. The predominant cells are nucleated red cells of the normoblastic type.

As a result of this acceleration of the process of red cell production there is a tendency for the cells hastily formed to be imperfect and defective in various ways, and for the cells to be hurried into the circulation before they are fully matured. Defective cell formation is seen in abnormalities in size, shape, and color. The cells tend to vary in diameter more than normally, many of them are distinctly smaller than normal and are also thinner and paler. The average amount of hemoglobin per cell is below normal, so the color index is below 1.0. There may be occasional deformed cells

(poikilocytes). There is an increase in reticulocytes, and in polychromatophilic red cells, and there may be an occasional normoblast.

As these newly formed cells reach the circulation there is a rise in the red cell count and hemoglobin. However, as many of the cells are small and have a low hemoglobin content, the red count rises faster and reaches normal much before the hemoglobin. As the lack of hemoglobin is approximately compensated for, the rate of cell formation lessens, and the newly formed cells tend to be more nearly normal, are richer in hemoglobin, and are more matured before reaching the circulation. As these more normal cells gradually replace the imperfect undersized pale cells, the microscopic appearance of the film becomes normal, and the color index rises to 1.0. Quite frequently the reparative process is somewhat overdone, and during convalescence the red count, and to a less extent the hemoglobin, may reach figures somewhat above normal.

Along with the acceleration in red cell production there is also increased formation of leucocytes and platelets. There is a neutrophilic leucocytosis, usually moderate in degree, but exceptionally it may be high, and rarely there may be considerable numbers of myelocytes, and even a few myeloblasts present.

The degree of reaction and the time required for restoration to normal depend on the rapidity of the hemorrhage, as well as the total volume of blood lost, and on the reacting power of the individual. Children and old individuals stand bleeding badly. Pregnant women stand it best, partly perhaps be-

cause of their increased blood volume. Loss of half or more of the total blood volume at one time is usually acutely fatal, unless quickly replaced by transfusions. Relatively small hemorrhages may cause appreciable changes in the blood, even quantities less than 100 cc. In a healthy young adult after a severe hemorrhage which is followed by an effective reaction on the part of the marrow, evidences of regeneration (defective cells, immature cells) may be found within 24 hours. The height of the reaction, with minimum color index and maximum abnormalities in the cells, is likely to be found about 8 to 10 days after the hemorrhage. Complete restoration to normal may be reached after about 30 days. Individual cases will show great variations from these figures, particularly in the presence of disease.

CHRONIC POSTHEMORRHAGIC ANEMIA

The result of repeated hemorrhages depends in part on the total volume of blood lost, but even more on the intervals between the hemorrhages. Large repeated hemorrhages may be surprisingly well borne if the interval between is sufficient for complete regeneration to occur. On the other hand, serious results will follow if the intervals are too short for this, even though the total volume of blood lost is materially less. The most marked changes in proportion to the total amount of blood lost occur in those cases with consecutive daily losses of relatively small amounts of blood, such as may occur from hemorrhoids or from the ulcerated surface of carcinomata of the digestive tract.

The morphological changes in the blood during the earlier stages of the process are much like those described as occurring after acute hemorrhage except that they are likely to be quantitatively more pronounced. The majority of the cells are usually distinctly below normal in diameter and quite strikingly pale. The centers may be almost colorless, so that the cells appear like rings, the so-called pessary forms. There is usually also a small number of cells which are as dark as normal or even darker. Anisocytosis becomes marked, there are many microcytes, and some cells which are normal or distinctly above normal in size. These macrocytes are usually pale, and often present an appearance of being swollen or as some have described it, "water-logged." Immature cells appear in varying numbers and sometimes are quite numerous. There is usually a neutrophilic leucocytosis, and an increased number of platelets. During this stage there is active hyperplasia of the red marrow, which, however, is unable to keep pace with the continued loss of blood. The hemoglobin and red cell count fall, and the color index becomes low, as does the volume index. The concentration of hemoglobin in the red cell substance tends to fall, so that the saturation index also becomes low, lower than in other types of anemia except possibly chlorosis.

Exhaustion of the marrow eventually occurs if the hemorrhages persist. Histological examination no longer shows evidence of hyperplasia, but rather a reduction below the normal in the rate of cell proliferation. The marrow becomes aplastic. These changes in the marrow are reflected by correspond-

ing changes in the blood picture. There is first of all a disappearance and persistent absence of immature red cells. There are few if any reticulated red cells, no basophilic red cells, and no normoblasts or cells containing nuclear particles. There is usually also a slowing of the production of leucocytes and platelets, so that there is a leucopenia, affecting especially the neutrophiles, and a subnormal platelet count. Because these blood changes have been found to accompany an aplastic bone marrow, a condition showing them is sometimes termed an aplastic secondary anemia, or better an aplastic stage of a secondary anemia. They have a grave prognostic significance.

In rare cases, with retarded red cell formation, such cells as are formed seem to be formed in a more leisurely manner, and individually are less strikingly abnormal. They may show less variation in size, and less pallor. While the hemoglobin and red count continue to fall, often to very low figures, the color index may tend to rise toward normal. However, a low color index is the characteristic feature of the great majority of cases of chronic hemorrhagic anemia throughout their course.

Very extreme degrees of anemia may develop after repeated hemorrhages. A hemoglobin of 20 per cent and a red count of 2.0 millions or less is not unusual, and occasionally they may be reduced to half these figures. The color index is often reduced to 0.5, and rarely even lower.

The anemia which develops in hookworm infestation has the characteristics of a chronic posthemorrhagic anemia. In most cases an appreciable anemia

is present. This is usually moderate in degree, but in severe cases the red cell count may fall below 1 million.

SYMPTOMS OF ANEMIA

The symptoms due to anemia *per se* are best studied in the case of anemia following simple hemorrhage, since here they are not confused by those caused by some underlying disease process. The symptoms of shock and collapse immediately following an acute hemorrhage are manifestations of circulatory failure due to loss of fluid from the blood vessels, and are not attributable to lack of hemoglobin. Unless the hemorrhage is quickly fatal these symptoms usually subside within a few days, as the diminished blood volume is restored, provided further bleeding does not occur. Later symptoms depend on lack of hemoglobin.

Slight grades of anemia are usually symptomless. Even a moderate grade of anemia may cause little more than a feeling of weakness and fatigability and loss of energy. Marked anemia causes definite and characteristic symptoms. The more important and constant are:

(1) *Circulatory system:*

On exertion there is breathlessness, tachycardia, palpitation, and sometimes arrhythmia. There is frequently a disagreeable throbbing in the peripheral vessels. Without evidence of dilatation of the heart, systolic murmurs are usually present at the apex, over the precordium, and at the pulmonic area. The murmur may be fairly intense, but it is generally not well transmitted to the axilla,

and there is usually no significant change in the character of the sounds themselves. Diastolic murmurs are rare. There may be edema of the ankles, and less often serous effusions. In cases of severe chronic anemia of long standing (*e.g.*, hookworm infestation) there may occur a dilatation of the heart, with "relative insufficiency" of the orifices, and all the symptoms and physical signs of chronic myocardial insufficiency may appear. Substantially a complete recovery from this condition may occur if the cause of the anemia can be removed.

(2) *Central nervous system:*

Headaches are common. Frequently there is dizziness and a feeling of faintness, especially on bending over or on sudden change of position, which may go on to actual syncope. There is frequently roaring in the ears, and black spots before the eyes. There is often insomnia, restlessness, irritability, emotional instability, lack of concentrating power, and incapacity for mental as well as physical exertion.

(3) *Gastrointestinal system:*

Loss of appetite and vague digestive discomforts are common. There is often constipation. Less often there is marked abdominal discomfort, flatulence, nausea, and vomiting.

In profound degrees of anemia, especially if the hemoglobin is much below 40 per cent, these symptoms are present in exaggerated degree. Practically every function of the body is more or less affected. There are menstrual disturbances (most often amenorrhea) in the female, and loss of libido and potentia in the male. There is often albuminuria,

with some impairment of renal function, which may be shown by nocturia and lessened concentrating power. In the severest cases, even without much cardiac dilatation, weakness and faintness may become extreme, and syncopal attacks may occur on raising the head, turning in bed, or even spontaneously. In some cases anemia may be the immediate cause of death.

Occasionally, however, patients with fairly marked degrees of anemia may be surprisingly free from symptoms, and a patient with only 30 to 40 per cent of hemoglobin may be capable of a considerable degree of physical activity with little discomfort.

TREATMENT OF ANEMIA

For the collapse (shock) following *acute hemorrhage*, transfusion of blood is the only dependable remedy. If the condition is critical, prompt transfusion will often be life-saving, but it should be carried out at the earliest possible moment. In such a situation one should use a known safe universal donor, if available, without waiting for the preliminary tests for compatibility which in a less urgent emergency should be regarded as indispensable for complete safety. One pint or two pints (from two donors) should be given. Surgical measures should be taken if practicable, to stop the bleeding. The patient should be kept warm, but not to the point of sweating, and should be kept quiet and comfortable, by the use of morphia, if necessary, unless some surgical complication contraindicates its

use. Stimulants and other drugs are practically useless.

If transfusion is impossible one should give 500 cc. of fluid intravenously, either as a 6 per cent solution of gum acacia or as physiological salt solution. These are both far inferior to transfusion. The gum solution is the more effective as it is retained much longer in the vessels, but it is prone to cause disagreeable reactions. Salt solution is rapidly excreted and its effect is very transient. It must be repeated in a few hours. Hypodermoclysis with salt solution may be tried in default of anything better, but it is relatively ineffective, if ever really life-saving.

If the patient's condition is less critical, and particularly if the site of the hemorrhage is not accessible to surgical interference, it may be advantageous to delay the performance of a transfusion for a few hours in order to take advantage of the favorable effect of the lowered blood pressure in stopping bleeding. Preparations should be completed for transfusion, and the patient carefully watched. The blood pressure is the best guide, and with a progressive fall in the systolic pressure to a point below 90 transfusion is usually needed. In case of doubt it is wiser to transfuse. The number of transfusions that may be given (if bleeding continues) is limited only by the number of available donors, and they may be repeated as frequently as the condition of the patient may call for them.

For the subsequent treatment of the anemia that follows acute hemorrhage in an otherwise healthy individual general hygienic measures alone usually

suffice. Rest in bed, if possible in the open air and sunlight, and a well-balanced diet are the essentials. Under these measures alone blood regeneration usually proceeds with rapidity.

Diet. Whipple and his associates[1] have studied carefully the effect of various food stuffs in accelerating hemoglobin formation in dogs made anemic by repeated bleedings. They found that the most effective foods were mammalian or bird liver, kidney, or gizzard. Next in order, but much less effective, were red muscle meats, other glandular meats, and certain fruits, especially apples, peaches, and prunes. Greens were less effective, but it is not improbable that they might be more effective in man than in the carnivorous dog. Cereals and milk products were poor, and fish liver was worthless in this respect. There is as yet no satisfactory explanation for the marked variation in the effectiveness of these different foods. Iron content is one important factor but not the only one. Whipple suggests that it may depend on other inorganic elements present.

These experiments confirm previous clinical observations that it is desirable to include in the diet of anemic individuals liberal amounts of red meats, green vegetables, and fruits. They also emphasize the great value in the diet of liver and kidney, the effectiveness of which had not previously been realized.

Liver. The mode of action of liver in promoting hemoglobin formation in secondary anemia is not well understood, and at present its use is based

[1] Whipple, G. H., *Am. Jour. Med. Sci.*, 1928, lcxxv, 721.

on empirical observation. Liver has no such specific effect in any form of secondary anemia as it has in pernicious anemia and the allied megalocytic anemias. In many types of anemia it is quite ineffective (hemolytic jaundice, acute aplastic anemia, sickle cell anemia, the leucemias). The conditions in which it is more likely to be effective are cases of posthemorrhagic anemia, and some forms of chronic anemia of an aplastic type which are probably associated with dietary insufficiency. However, until its limitations are more definitely established it will be desirable to give liver a thorough trial in any resistant form of secondary anemia. Whole liver should be given in a quantity of not less than half a pound a day for several weeks before its ineffectiveness can be regarded as established. Liver extract is not usually effective in secondary anemia, and the mechanism of the action of liver must, therefore, be essentially different from that in pernicious anemia. However, Minot has found that liver extract may be effective if supplemented by a relatively small amount of whole liver.[2]

Liver might benefit secondary anemia by means of its iron content, by the vitamines it contains, especially vitamines A, D, and E, by its content in proteins and other nutritive substances, and probably also by other unidentified substances which stimulate the marrow. Minot believes that liver has a special effect in stimulating the formation of red cells (stroma), whereas iron more specifically stimulates hemoglobin formation. The simul-

[2] Minot, G. R., *The Thayer Lectures*, Baltimore, 1929.

taneous administration of iron and liver may be more effective than either one alone.

Iron. The value of administering iron in the treatment of posthemorrhagic anemia has been disputed. Its value was first seriously questioned by Whipple, who showed that dogs recovered from an experimental posthemorrhagic anemia as rapidly on a suitable diet (rich in liver) without medicinal iron, as when iron was administered. There is no doubt that the iron in the food constitutes a readily utilizable and usually adequate supply for hemoglobin formation. Subsequent work, such as that of Riecker,[3] seems to show that the usefulness of additional medicinal iron depends on the degree to which the reserve store of iron in the body has been depleted. A single acute hemorrhage had little effect on this reserve, but repeated bleeding might exhaust it. In such depleted animals recovery from the anemia was slow, and it was definitely accelerated by iron administration, more so than by liver feeding alone. This accords well with clinical experience. In most cases of acute hemorrhage recovery from the anemia proceeds with such rapidity that iron administration can have little measurable effect on it. In the more severe anemia following chronic hemorrhage the beneficial effect of iron therapy is more evident.

While medicinal iron can often be dispensed with, it is still desirable in many cases of secondary anemia. Iron may be given as Blaud's pill, as reduced iron, or perhaps best as a soluble ferric salt, such as ferri et ammonii citras. There is no evidence

[3] Riecker, H. H., *Jour. Clin. Investigation*, 1927, v, 141.

that medicinal "organic iron" preparations are more effective than these simple inorganic salts. It should be given by mouth. There is no advantage in hypodermic or intravenous administration. The dose, however, must be adequate. While small doses such as 0.2 grams three times a day may suffice, in some cases as much as 1.0 gram or more three times a day may be essential to secure improvement. This point, recently emphasized by Minot, was clearly recognized by the older clinicians in the treatment of chlorosis.

Such quantities of iron are vastly greater than the amount which could possibly be utilized directly in hemoglobin formation. Probably only a small part of the iron is actually absorbed, but it is probable that the absorption of quantities of iron larger than those which enter directly into the hemoglobin molecule may serve a useful purpose by directly stimulating the marrow, as it clearly does in cases of chlorosis. Support for this view is found in the occasional occurrence after iron administration in secondary anemia of a reticulocyte crisis, such as follows the administration of liver in pernicious anemia.

Accessory Factors. While it is essential that an adequate quantity of iron be administered, or available, for satisfactory recovery from anemia, undoubtedly other factors are also necessary to secure its effective utilization. Just what these other factors are has not been definitely determined, but several interesting hypotheses have been suggested by recent experimental work in animals. Under certain conditions the particular preparation of iron

used may be important. Thus McCollum and his associates[4] have shown that rats on a certain restricted diet otherwise satisfactory will thrive if iron is administered as ferric citrate, but will sicken and die if it is given as the sulphate. They also found that rats on this diet receiving iron as ferric sulphate would recover and thrive if they were given in addition a little wheat germ oil. This oil contains among other things vitamine E, the vitamine shown by Evans to be essential for the normal sexual function in the rat. McCollum believes that this vitamine is concerned also with the process of iron assimilation. It is present in liver fat, and could be a factor in the favorable therapeutic effect of liver in the diet.

Hart, Steenbock, Waddell, and Elvehjem[5] have shown that rats which were made anemic by a milk diet would not recover under the conditions of their experiments if inorganic iron was administered as chemically pure ferric sulphate or citrate. The rats would recover, however, if the same quantity of iron was given in the form of an ashed residue from beef liver, lettuce leaves, or yellow corn. The effectiveness of iron in such ashed material presumably must be due to some other inorganic substance also present in the ash. This substance they believe to be copper, which they demonstrated in minute amount. They found that a similar minute amount of a pure copper salt administered with the iron would also

[4] Simmonds, N., Becker, J. E., and McCollum, E. V., *Jour. Am. Med. Assn.*, 1927, lxxxiii, 1047.

[5] Hart, E. B., *et al.*, *Jour. Biol. Chem.*, 1928, lxxvii, 797.

bring about recovery. They believe that copper plays a rôle in hemoglobin formation.

The results of these animal experiments do not now permit of generalizations which may be applied directly to disease in man. They do serve to show that hemoglobin formation is really a complex process, and that the treatment of anemia involves more than merely the liberal administration of iron. They indicate the need of substances which do not enter directly into the composition of the hemoglobin molecule. From the practical standpoint they emphasize the importance of a diet which is varied in composition, and rich in vitamines and mineral salts, in order to insure as far as possible that an adequate quantity of these as yet unidentified adjuvants may be secured. Whole liver is a valuable source of such substances.

Arsenic may also be given for its stimulating effect on the bone marrow. Its effectiveness in post-hemorrhagic anemia is less clearly demonstrated than in pernicious anemia. Arsenic may be given as Fowler's solution by mouth, or as sodium cacodylate hypodermically.

If the degree of anemia is marked, or improvement is slow, blood *transfusion* is of great benefit and should be resorted to without hesitation. It is especially desirable in patients with associated chronic disease. A single transfusion of a pint will often suffice to initiate a steady satisfactory rate of improvement. In some cases, especially of chronic anemia of long duration, repeated transfusions may be desirable. To obtain a rapid increase in hemoglobin with the least reaction on the part of the

patient, as in anemia of great severity, the optimum interval between transfusions is 3 or 4 days. To secure the maximum stimulating effect of the transfusion an interval of about ten days is probably preferable.

CHAPTER IX

ANEMIAS DUE TO BLOOD DESTRUCTION

ACTUAL solution of circulating red cells with liberation of hemoglobin into the plasma in notable amounts is a rare phenomenon. It does occur in a few conditions, however. Whenever a considerable amount of hemoglobin is liberated abruptly into the blood (hemoglobinemia), a portion of it is rapidly excreted in the urine (hemoglobinuria). This occurs when 1/60 or more of the red cells are suddenly destroyed (Ponfick and Stedelman). Hemoglobinuria occurs in the following conditions:

(1) Paroxysmal hemoglobinuria.
(2) Reactions following the transfusion of incompatible blood.
(3) In certain infections:
Malaria (black-water fever).
A few isolated cases have been reported in other human infections: gas bacillus gangrene, sepsis, typhoid fever, scarlet fever. Texas fever of cattle and piroplasmosis of horses.
(4) Rarely after certain poisons: arsenurated hydrogen, potassium chlorate, snake venom, saponin, phenylhydrazin.
(5) Rarely after extensive burns.

PAROXYSMAL HEMOGLOBINURIA

This is a rare disease characterized by recurring brief paroxysms of chills, fever, pains in the lower back, paresthesias in the extremities, and followed by the passage of high-colored urine containing free hemoglobin or methemoglobin, but no intact red blood cells. The attacks are brought on in susceptible individuals by some definite exciting cause, usually exposure to cold. A patient can often bring on an attack at will by immersing a hand or foot in cold water. In rare instances other precipitating factors may be concerned, such as severe muscular exertion. A paroxysm usually begins abruptly and lasts from 2 to 4 hours. If severe there is great discomfort, prostration, often vomiting and diarrhea, and abdominal pain which may be severe. The temperature may reach 103. There may be vasomotor disturbances of the extremities resembling Raynaud's disease, with numbness and tingling, cyanosis, edema, purpuric blotches, and even gangrene. The blood serum during an attack is more or less deeply tinged with hemoglobin. Some of the products of hemolysis are toxic, and are doubtless the direct cause of the symptoms mentioned. The urine varies in color from a light pink to a deep black, depending on the quantity of blood destroyed. Not all the hemoglobin liberated is excreted. Much is retained and split up in the body, as in the course of normal blood destruction. After the attack there is increased bilirubin in the serum, urobilin in the urine, and slight jaundice. The paroxysms are followed by more or less severe anemia. The red cell

count may fall to 1 million after a single paroxysm (Morawitz). Death during a paroxysm is rare, and recovery is usually rapid. Relapses are practically invariable, and in many cases the susceptibility to attacks increases if paroxysms are permitted to occur.

The mechanism of the hemolysis was first worked out by Donath and Landsteiner. There is a true hemolysin in the blood of these patients, which is capable under suitable conditions of dissolving their own red blood cells (as well as those of other normal human beings); that is, it is an "autohemolysin." It has the complex structure characteristic of the specific "immune" hemolysins; that is, it is made up of a stable constituent ("amboceptor") and a labile constituent ("complement"). Like other hemolysins, it can attack the cells only after the stable constituent (amboceptor) has combined with them, but unlike most other hemolysins, this amboceptor can combine with the cells only at low temperatures. If the blood has been chilled so that the amboceptor has combined with the cells, the complement in the serum will bring about rapid hemolysis of the cells when the temperature is again raised. This phenomenon of fixation of the hemolysin only in the cold obviously is true in the body, as well as in the test tube, and explains the effect of chilling in precipitating an attack. It is often referred to as the Donath-Landsteiner phenomenon. No significant abnormality in the red cells has been demonstrated.

The *etiology* of the disease is still under discussion, but it is generally believed to be a late manifestation of syphilis. A large majority of the cases

of paroxysmal hemoglobinuria give a positive Wassermann reaction, and many of them give a history of syphilis, or show clinical evidence of this infection. Although the percentage of syphilitics who develop paroxysmal hemoglobinuria is extremely small, in a fair proportion of cases of late syphilis (5 to 10 per cent) the blood has been shown to contain the peculiar hemolysin, although the patients had never had clinical symptoms of paroxysmal hemoglobinuria. The hemolysin is rarely if ever found in nonsyphilitics. Some predisposing constitutional peculiarity of the individual must be assumed in addition to syphilis.

The *diagnosis* can be made from the description of the attacks, and by demonstrating that the freshly voided urine contains hemoglobin in solution, and not intact red blood cells. Conclusive proof is furnished by demonstrating the presence of the peculiar hemolysin.

Treatment is not regularly effective in removing the tendency to the attacks. A thorough course of antisyphilitic treatment should be given. This has not usually caused a disappearance of the hemolysin from the blood, and in many reported cases has not stopped the paroxysms. Some cases have been reported, however, in which a symptomatic cure was obtained (Jones,[1] etc.). It is important to avoid exposure to cold and other conditions which have been found to precipitate attacks, as otherwise susceptibility tends to increase. There is no way of influencing the course of a paroxysm, once it has

[1] Jones, B. B. and C. M., "Paroxysmal hemoglobinuria," Nelson's *L.L.L.Med.*, iv, 173.

been initiated, except to keep the patient warm. For the resulting anemia, if severe, the usual measures suitable for the treatment of a secondary anemia should be adopted.

MANIFESTATIONS OF ACCELERATED BLOOD DESTRUCTION

In the great majority of cases in which direct toxic injury to the cells occurs, the damaged cells are removed from the circulation before hemoglobin is liberated and before any change can be made out in their microscopic appearance. There is in such cases a great exaggeration of the normal process of red cell destruction by the reticulo-endothelial cells of the spleen and other organs. As previously described, the pigment liberated on disintegration of the hemoglobin molecule (at least in part) is converted in these tissues into bilirubin. This is carried in the plasma to the liver, where it is taken up and excreted in the bile. If the liver function is not disturbed, and if blood cell destruction is not too rapid, the bilirubin will be removed from the blood as fast as it is formed and only traces will be found in the plasma.

If blood destruction is accelerated (or if the liver is injured), the liver may be unable to keep pace with this accelerated formation of bilirubin. The latter will then accumulate in the plasma, imparting to it and to the serum a distinct yellowish color, the depth of which in general is roughly proportional to the rate of blood destruction. If this hyperbilirubinemia lasts for any appreciable time the tissues become more or less jaundiced. In some

cases the jaundice is marked, although there is rarely the greenish tint that may be seen in many cases of chronic obstructive jaundice. In most cases the tinting of the tissues is relatively slight, and the color of the skin and scleræ is a pale lemon yellow, rather than the orange tint of obstructive jaundice. Although this difference in tint has been emphasized as of diagnostic importance, and is often quite evident, the difference is only a quantitative one, as the color has been shown to be due to bilirubin.

In interpreting the significance of jaundice of this type one must remember that it may develop as a result of liver injury without notable increase in blood destruction. Accumulation of bilirubin in the plasma may be due to inadequate removal, as well as to increased formation.

The presence of bilirubin in the serum may best be demonstrated by the Van den Bergh test. Its quantity is most conveniently estimated by determining the icteric index of the serum. Both are very simple procedures. (See page 290.)

Unless there is bile duct obstruction, no bile, or traces only, will appear in the urine. The jaundice is acholuric. The urine becomes dark colored, but this color is due to other pigments, especially to urobilinogen and urobilin. The usual explanation offered for this is that urobilinogen (stercobilin) is formed in the intestine by bacterial action, in large quantity from the increased quantities of bilirubin present, and is then absorbed into the blood and excreted into the urine. Whipple, however, questions the absorption of urobilinogen from the intestine, and thinks that, like bilirubin, it may be formed

in the tissues and enter the blood directly. In either case the urobilinogen and urobilin in the duodenal contents and feces are markedly increased, and increased quantities are present in the urine.

To *summarize*, the distinguishing features of an hemolytic anemia in its active stages are, in addition to the anemia, the development of jaundice, usually slight in degree, with a lemon yellow discoloration of the skin and scleræ; the excretion of a high-colored urine which contains urobilinogen and urobilin but no bile; an increase in these pigments in the feces and duodenal contents; and an increase in the bilirubin in the serum, as shown by its yellow color (high icteric index) and by a positive (indirect) Van den Bergh reaction. In such a patient at autopsy there will be an increase in the iron content of the liver and spleen. These phenomena appear distinctively only during periods of rapid blood destruction. If the process is slow, they will be absent or detectable only by a careful quantitative test for bilirubin in the serum.

Occurrence. These phenomena are observed in most marked degree in the acute phases of familial hemolytic jaundice, of primary pernicious anemia, and of sickle cell anemia, all distinct diseases which require special consideration. They may also be met with during the absorption of extensive extravasations of blood, and in some cases of anemia following acute infections and intoxications. In these latter conditions it may be difficult to determine to what extent these phenomena are due to increased blood destruction, on the one hand, or to liver injury, on the other.

The *microscopic appearance* of the blood in these cases of secondary hemolytic anemia is not definitely distinctive. In general the changes resemble those described in posthemorrhagic anemia. If the injury to the marrow has not been too severe, there is a tendency for the newly formed cells to be less abnormal, and in particular to be more adequately supplied with hemoglobin, so that the color index is relatively less reduced. This is probably because the iron and other important cellular constituents are largely retained in the body after intracorporeal blood destruction, whereas after hemorrhage they are lost and there may be a serious dearth of these raw materials as a result. In some types of hemolytic anemia (*e.g.*, lead poisoning) there are many basophilic staining cells and other types of immature red cells. In some cases the cells may be as large as normal, or larger, and of good color, so that the color index will be 1.0 or even higher, and the blood may show some resemblance to that of pernicious anemia.

Treatment of the underlying cause of the anemia is of primary importance. Transfusions may be of value as a supporting measure, but are of little avail during periods of rapid blood destruction.

CHAPTER X

ANEMIAS DUE TO DEFICIENT BLOOD FORMATION (APLASTIC ANEMIAS)

IN MOST types of secondary anemia, except that due to hemorrhage, the anemia is due more to injury to the marrow, with retardation of the process of new cell formation, than to an accelerated destruction of red cells after they have entered the circulation. That is, the anemia is in large part the result of bone marrow insufficiency. The normal marrow is capable of replenishing lost cells with astonishing rapidity. The deficiency in the activity of the marrow as a rule is only relative. In most cases the blood shows evidence of active cell regeneration, such as the presence of imperfect and immature red cells, of a leucocytosis, and of an increase in platelets. In some cases, however, these evidences of regeneration are lacking, and the blood may present those features already described as characteristic of an inactive or aplastic marrow. There is an absence of immature red cells; of reticulated and polychromatophilic red cells, of normoblasts and cells with nuclear particles. Although there is no evidence of accelerated blood destruction, the red cell count and hemoglobin fall rapidly. The changes in the appearance of the individual red cells are often relatively slight. Anisocytosis may be only

moderate in degree, and the color of the cells may be nearly normal, so that the color index is but little less than 1.0. There is a leucopenia and a reduced platelet count. That such a condition of the blood indicates a profound marrow injury or insufficiency is shown by an absence of hyperplasia of the marrow at autopsy.

Occurrence. Such an aplastic condition may develop during the terminal stages of a great variety of secondary anemias, as well as in pernicious anemia. In some cases of anemia these aplastic features are prominent almost from the start. Such cases are met with most frequently in the following conditions:

(1) Some severe cases of infection.

(2) Chronic intoxication with blood poisons, such as benzol and trinitrotoluol.

(3) Excessive exposure to X-ray or radium.

(4) The terminal stages of some myelophthisic anemias.

(5) Idiopathic (or acute, or primary) aplastic anemia.

Idiopathic (Primary) Aplastic Anemia has been studied especially by Cabot.[1] These rare cases occur chiefly in adolescents and young adults, more commonly in females. The cause of the disease is unknown. The onset is usually insidious, with weakness, fatigability, and pallor. These symptoms progress rapidly without any tendency to remission. There is often fever, and sooner or later, associated with the reduction in platelets, there develops

[1] Cabot, R. C., Osler's *Modern Medicine*, Ed. 1, iv, 637.

a symptomatic purpura hemorrhagica, with profuse bleeding. Death invariably occurs within six months, and not rarely within a few weeks.

The blood shows in extreme degree the characteristics above outlined. Noteworthy are the extreme degree of leucopenia and thrombopenia and the relatively trivial changes in the appearance of the individual red cells, despite the severe anemia. At autopsy the marrow is aplastic.

Treatment is unavailing. Transfusion may be of some temporary benefit.

Differential diagnosis. It is not certain that primary aplastic anemia constitutes a disease entity, but the clinical picture suggests it. It must be differentiated from:

(1) Aplastic types of anemia due to some demonstrable cause.

(2) Pernicious anemia in an aplastic stage.

(3) Essential thrombopenic purpura. In this disease the anemia is the direct result of hemorrhage, and is of the posthemorrhagic type, with a low color index. Extreme leucopenia is rare, although the platelets are much reduced. In aplastic anemia the anemia is the primary feature, it precedes bleeding, and, at least at the start, it is out of proportion to the degree of blood loss.

(4) Acute leucemia with an "aleucemic" (that is, leucopenic) blood. The minute study of the white cells will usually determine the diagnosis.

Cases occur in which certain differentiation cannot be made between primary aplastic anemia and the two last mentioned diseases. (See Aleukia.)

It is important to remember that the term "aplastic anemia" is used to indicate (1) the rare clinical entity of unknown etiology above described, and (2) more commonly and of greater importance a clinical and hematological syndrome due to exhaustion of the marrow from some external and usually demonstrable cause. This syndrome may appear early in the course of a fulminatingly acute infection or intoxication, or it may appear at a later period during the course of any severe chronic anemia. If the etiology in such a case cannot be determined, it is quite proper, for want of any more precise diagnosis, to call the condition *an* aplastic anemia, bearing in mind that in so doing one is describing rather than identifying the disease.

Treatment of the secondary aplastic anemias must be directed primarily to removal of the underlying cause of the anemia, and the prognosis depends entirely on the effectiveness of such measures. Otherwise only palliative and supportive measures are available, of which transfusion is the most important. Transfusions should be used freely, particularly if the degree of anemia is marked, but they cannot be expected to cure the underlying cause of the anemia.

CHAPTER XI

SPECIAL FORMS OF SECONDARY ANEMIA

ANEMIAS SECONDARY TO INFECTIONS

THE severe anemia that may develop rapidly in *acute sepsis*, especially the streptococcal forms, has already been mentioned. The extreme example is the classical case of Grawitz, where in puerperal sepsis (but complicated by hemorrhage) the red cell count fell from normal to 300,000 within 24 hours. Even in the absence of bleeding the count may fall to 1.5 millions or less within a very few days. Usually the fall is slower and much less marked, but a rapidly progressive, marked anemia is a fairly common accompaniment of severe acute infections. This is also seen in some cases of scarlet fever, acute rheumatic fever, typhoid fever, and malaria.

Severe anemia may develop, more slowly, as a result of chronic infections. Among these may be mentioned *chronic pyogenic infections*, such as chronic osteomyelitis, severe oral sepsis, and the severe forms of chronic infectious arthritis. Severe anemia is practically constant in the later stages of *chronic bacterial endocarditis* (streptococcus viridans) and may dominate the clinical picture. It is usually slowly progressive, and the hemoglobin may fall to 40 per cent or under. *Syphilis* in the secondary stage often causes an anemia of moderate

degree. In the tertiary stage the blood is often normal, but a severe anemia occasionally develops.

Tuberculosis often causes a relatively slight degree of anemia of the secondary type, with a moderate reduction in hemoglobin, and a normal or slightly reduced red cell count. In the absence of hemorrhage, a severe anemia is rare and is not at all characteristic of the disease. Even in advanced cases with marked emaciation the degree of anemia is usually slight unless complicating secondary infection is present.

Hodgkin's disease (if it be an infection) in its early stages causes little change in the blood. In the late stages a severe anemia of the secondary type develops. The count may fall to 1.5 millions, the hemoglobin to 20 per cent.

ANEMIAS DUE TO CHEMICAL POISONS

Among the chemical poisons which most often cause anemia may be mentioned the heavy metals, such as lead, mercury, arsenic, and radioactive substances; benzene (benzol) and a great variety of its chemical derivatives; the nitrites, iodine, and potassium chlorate.

Lead Poisoning causes the best known and most important of the anemias in this group. In cases of acute lead poisoning there is rapid blood destruction, with an increase in the bilirubin in the serum, and the other phenomena of an acute hemolytic anemia. The red cell count and hemoglobin fall rapidly, but in human cases do not often reach extremely low figures. The color index is a little below 1.0. There is usually a prompt and marked

response on the part of the bone marrow, as shown by the appearance of imperfectly formed red cells, and of immature red cells of every description, usually in large numbers. In the more common cases of chronic lead poisoning similar changes appear more gradually.

Aub and his associates [1] have studied in detail the *toxic action* of lead *on the red cells*. They found both in vivo (man and rodents) and in vitro that "leaded cells" show evidence of changes in the physical condition of their superficial layer, as a result of which they become somewhat shrunken and heavier, are relatively impervious to water, show an increased resistance to the solvent action of hypotonic salt solution, but are brittle and much more susceptible than normal to mechanical injuries. This latter fact, they think, may be the immediate cause of the rapid destruction of the cells in the circulation. These peculiar changes were not caused by any of the other blood poisons which they studied.

The most characteristic feature of the blood in lead poisoning is the presence of relatively large numbers of *stippled red cells*. So striking is this phenomenon in many cases that it has come to be regarded by many, incorrectly, as practically diagnostic of lead poisoning. Such cells may be present in any type of anemia in which active regeneration is taking place. Even in apparently normal individuals, an isolated stippled cell may be found under certain conditions, as after eating "blutwurst," and conceivably after other harmless stimuli. Never-

[1] Aub, J. C., "Lead poisoning," *Medicine*, 1925, iv, 1.

theless, the appearance of considerable numbers of these cells is highly characteristic and should always arouse a strong suspicion as to the possibility of lead poisoning. Their appearance in significant numbers is not absolutely constant, although practically so if any noteworthy anemia is present. Quite often they are sparse, and careful search may be required to find them.

Stippled cells are not only the most constant and characteristic sign of lead poisoning, but are usually the earliest manifestation. They may appear within 24 hours after acute poisoning, and within 3 or 4 days of casual exposure to lead in the industries.[2] They may occur in the absence of any anemia whatsoever, or of any other clinical evidence of poisoning. In German industries attempts have been made with some measure of success to utilize counts of stippled red cells in exposed workers as a test of individual susceptibility to lead poisoning, and as an indication for enforced change of occupation. The number which may be regarded as significant in this connection is variously stated from 100 to 300 per million red cells.

Stippled cells are invariably accompanied by diffusely basophilic staining red cells, and are usually outnumbered by them. The latter, however, are not as distinctive of lead poisoning. A careful search will usually reveal also a few normoblasts and cells with nuclear particles.

The degree of anemia does not necessarily parallel the severity of the other symptoms. With removal from exposure the anemia gradually subsides, often

[2] White and Pepper, Lambert in Osler's *System of Medicine.*

with considerable rapidity. Stippled cells usually persist until the hemoglobin returns to normal, often for many weeks.

Mercury is next in importance to lead among the metallic poisons. Anemia is much less constant and striking but may occur either in acute or chronic poisoning, or after therapeutic use of the drug. The anemia is usually moderate, and the hemoglobin is more reduced than is the red cell count. A sharp fall of 5 to 20 per cent in hemoglobin may follow the administration of a large dose of mercury, particularly in an untreated syphilitic. This was regarded at one time as diagnostic of syphilis (Justus), but it is inconstant and may occur in other conditions. The hemoglobin usually rises to its former level within a short time, even if the administration of mercury is continued, but in exceptional cases it may fall progressively and necessitate a change in treatment.

Among the *Benzene Compounds* which are of the greatest practical importance may be mentioned benzene itself (benzol), phenol, aniline, nitrobenzene, trinitrotoluol, phenylhydrazin, and acetanilid and related drugs.

The toxic action of benzene on the bone marrow is highly selective. It is manifested first and most intensively on the granular leucocytes. A marked leucopenia develops, due to a reduction of the granular leucocytes. In marked cases these cells may practically disappear from the blood. In such cases the total number of lymphocytes is also much reduced, although their percentage is high. The total leucocyte count may fall as low as 400 per c.mm.

Next in order of susceptibility to this poison come the platelets. In all but the mildest cases the platelets are decidedly reduced in number, often to 60,000 or less. With this fall there appear purpuric spots on the skin, hemorrhages from the mucous membranes, and the other symptoms of a thrombopenic purpura hemorrhagica.

The red cells are least affected. There is rarely any notable degree of anemia until profuse hemorrhage occurs. A severe anemia of the posthemorrhagic type then develops. The toxic action of the benzene on the erythropoietic tissue of the marrow is probably a contributing factor. Anatomically there is a marked aplasia of the leucopoietic tissue of the marrow.

Poisoning with benzene is a not uncommon form of industrial poisoning. We have observed a number of cases from canning factories in which poisoning occurred as a result of the inhalation of fumes from cleaning solutions which contained benzene. There is marked variation in individual susceptibility. Benzene poisoning should be suspected when the clinical picture of a purpura hemorrhagica is combined with a neutrophilic leucopenia.

Treatment. Removal from exposure suffices in the milder cases. There is no specific antidote. Death may occur from hemorrhage, or from intercurrent infection. In the more severe cases repeated transfusions of blood are urgently indicated, and are usually successful in checking the bleeding, at least for a few days. The local use of hemostatics, or the injection of horse serum or other coagulants is generally futile. The outlook is unfavorable in

cases with extreme leucopenia, but even severe cases may be saved by persistent transfusions.

Acetanilid may be taken as the type of those drugs which more specifically poison the red cells. Similar changes are caused by nitro-benzine, potassium chlorate, iodine, and phenylhydrazin. These drugs cause a conversion of hemoglobin into methemoglobin. If the *methemoglobinemia* is marked, the skin and mucous membranes assume a peculiar and characteristic dusky bluish color, frequently seen in victims of various proprietary nostrums which contain this drug. The diagnosis is established by spectroscopic examination. Such blood will show, in addition to the characteristic absorption bands of oxyhemoglobin, an additional band in the orange part of the spectrum. This must be distinguished from a similar band due to sulphhemoglobin, in cases of sulphhemoglobinemia.

A considerable amount of methemoglobin transformation may occur without notable red cell destruction. The anemia is slight in most cases. In severe cases, especially in acute poisoning, there may be a marked and rapid fall in both hemoglobin and red cells, with all the signs of an acute hemolytic anemia. The color index is about 1.0. There is usually a leucocytosis, and an increase in platelets. Anisocytosis and poikilocytosis become marked. A striking feature in some cases has been the presence of extremely large numbers of normoblasts and intermediates in the films (20,000 to 30,000 per c.mm., Stengel and White, Brown.)

Death from acetanilid poisoning is usually due to circulatory depression rather than to injury to

the red cells. The latter may be relatively slight. If injury to the cells is marked, in addition to the usual measures, oxygen inhalations by an effective method, and transfusions, should be tried.

The toxic action of phenylhydrazin is discussed in connection with the treatment of polycythemia. Chronic poisoning with these substances may give rise to a chronic aplastic type of anemia.

ANEMIA DUE TO NEOPLASMS

In a majority of the cases of malignant disease a notable degree of anemia eventually develops. In the earlier stages of the disease it is slight, and is often limited to a slight reduction in hemoglobin. In the more advanced stages there is usually an outspoken anemia which almost invariably is clearly of the secondary type. The color index is low, from 0.7 to 0.5 or less, and the red cells are pale and below the normal in average size. Anisocytosis may be marked, and polychromatophilic cells are usually numerous. An occasional normoblast may be present. There is usually a leucocytosis, and an increase in platelets. It is frequent to find the red cells reduced to from 3 to 4.5 millions, and the hemoglobin to from 40 to 70 per cent. Extreme reduction to 1 million red cells and 15 per cent hemoglobin may occur, but this is extremely rare except after hemorrhage.

In some cases hemorrhage is an important factor in causing the anemia. This is usually in the form of small repeated bleedings or continuous oozing rather than of copious hemorrhages. If it occurs from the gastrointestinal tract, it may be entirely

overlooked unless the stools are examined for occult blood. In many cases, however, a marked secondary anemia develops although hemorrhages can be excluded as a cause. The anemia in these cases is not necessarily proportional to the degree of emaciation, and it is not purely the result of malnutrition. It is usually attributed to the action of some "toxin," produced by the growth, although there is no direct proof of the existence of such a toxin. The action of this "toxin" appears to be primarily on the marrow, inhibiting cell formation (Naegeli). There is no direct evidence of abnormal cell destruction.

In some cases, particularly of carcinomata of the stomach or intestine which do not cause obstruction, anemia may be the earliest and the most striking manifestation of the disease. In such cases transfusion may be of the greatest benefit in prolonging the patients' period of activity and comfort.

There are two other groups of cases of cancer of relatively rare occurrence in which the blood changes differ from those described above.

(1) *Metastases to the bone marrow* (Myelophthistic anemia).

In many (but not all) of these cases in which the involvement is extensive the blood may show changes which indicate an intense local stimulation or irritation of the marrow tissue. The anemia is usually marked. The color index is about 1.0. Anisocytosis is marked. There may be numerous macrocytes which are usually round rather than oval, as in pernicious anemia. Polychromatophilic cells are abundant. The striking feature is the presence of numerous nucleated red cells, up to 300 or

more per c.mm. These are chiefly fairly mature normoblasts, with a smaller number which are distinctly immature, and an occasional cell (macroblast, Naegeli) which closely resembles the megaloblasts of pernicious anemia. There is an abundance of platelets and a neutrophilic leucocytosis, with often fairly numerous myelocytes and myeloblasts. If the involvement of the marrow is extensive, an aplastic type of anemia may develop in the terminal stages of the disease.

(2) In a few rare instances of cancer, chiefly of the stomach and intestine, without demonstrated bone metastases, a *macrocytic* type of *anemia* has been described which somewhat resembles pernicious anemia. An abundance of platelets and a leucocytosis usually make the differentiation possible. Because of their theoretical interest with relation to pernicious anemia, these cases have received more consideration in current hematological literature than their great rarity would warrant.

CHAPTER XII

CHLOROSIS

CHLOROSIS is a disease of unknown etiology, limited to females, characterized by an anemia of the secondary type with a very low color index, by the clinical symptoms which are associated with hemoglobin insufficiency, and by an extraordinarily prompt specific response to iron medication.

The disease is no longer met with in Baltimore, and is disappearing in other American and European cities to such an extent that it has become almost a clinical curiosity.[1]

In susceptible individuals the disease invariably appears shortly after puberty, although relapses are prone to occur later. This fact and the limitation of the disease to females have suggested the hypothesis that a causal factor is some endocrine disturbance, presumably an ovarian insufficiency (Naegeli, etc.). It is not improbable that dietary faults, possibly a deficiency of iron, or of some vitamine, may favor the development of chlorosis, but in general the disease seems to develop independently of any adequate external cause. In particular, chlorosis is not a result of tuberculosis, although it might be expected to predispose to tuberculosis. Bad hygienic conditions and an inadequate diet are not sufficient in themselves to produce

[1] Cabot, R. C., Osler's *System of Medicine*.

chlorosis, otherwise there would inevitably have been a recrudescence of the disease in Europe during the war.

The pathogenesis of the disease is not known. In all probability the fundamental feature is some disturbance of iron metabolism. The anemia in chlorosis is the result of inadequate blood formation. There is no evidence of increased blood destruction. The serum is pale, and the bilirubin content is low.

Symptoms. The onset is insidious, with feelings of weakness, fatigability, and usually dyspnea and palpitation. There may be edema of the ankles. Digestive disturbances are frequent: loss of appetite, epigastric discomfort, nausea, vomiting, and constipation. Gastric hyperacidity is the rule. Perversions of appetite are very common, such as craving for sweets, for sour foods, or for chalk, sand, and other inedible articles. Headache is common, as is tinnitus and black spots before the eyes. Menstrual disturbances are almost invariable, usually amenorrhea, or infrequent and scanty menses. In marked cases there is a tendency to venous thrombosis, especially in the legs, and death has occurred from pulmonary embolism (Naegeli) and from thrombosis of the cerebral sinuses (Osler). Bleeding does not occur.

On examination the striking feature is the pallor of the skin and mucous membranes, with maintenance of normal nutrition. However, as Cabot [2] says, "it takes the eye of faith" to see the green tint to which the disease owes its name. "Functional" cardiac systolic murmurs are the rule, and are some-

[2] Cabot, R. C., Osler's *Modern Medicine*, 3rd Ed., 1927, v, 59.

times associated with slight cardiac enlargement. Slight fever is frequent, as is a trace of albumin in the urine. The spleen, liver, and lymph glands are not enlarged.

The *blood changes* are highly characteristic, but not pathognomonic. The red cells are reduced moderately, usually to between 3 and 4.5 millions. The hemoglobin is relatively much more reduced. In Cabot's series, in over half his cases the hemoglobin was between 20 and 40 per cent, and the color index was between 0.3 and 0.5. This extreme lowering of the color index is the most striking feature of the blood in chlorosis, and is relatively rare in other forms of anemia. The red cells, on the average, are moderately undersized and strikingly pale. Occasional pale macrocytes occur, but anisocytosis and poikilocytosis as a rule are relatively slight. Polychromatophilic red cells and normoblasts are infrequent or usually absent in untreated cases. They may appear in large numbers during the period of regeneration that follows effective therapy. There is no notable change in the leucocytes or platelets. The lymphocytes are somewhat reduced.

Differential diagnosis. In view of the present rarity of the disease the diagnosis should be made with great circumspection. It is justifiable only in adolescent females, who show the characteristic blood changes described, and in whom the usual causes of a secondary anemia can be excluded. Anemia due to hemorrhage (especially menorrhagia) and to chronic infections must be kept in mind.

Treatment. All observers who have had any extensive experience with chlorosis are in agreement as to the prompt and striking benefit that follows adequate iron therapy in the great majority of cases. The best preparations are Blaud's pill (Osler, Cabot,[3] and many others) or reduced iron (Naegeli).[4] During the first week one 5-grain Blaud's pill is given three times daily after meals, during the second week the dose is increased to two pills, and from the third week on three pills are given three times daily. This dose is kept up for 6 weeks or more, and iron must be administered for several months, although the dose may be reduced. The rapidity of the improvement may be astonishing. Naegeli reports a case in which in 3½ weeks there was a rise from 3.3 millions red blood cells, and 32 per cent hemoglobin to 5.1 million red cells and 71 per cent hemoglobin. Cabot regards as usual an increase of 5 per cent in hemoglobin every ten days.

It is also important, and in some cases essential, to keep the patients in bed, to make sure that they eat liberal quantities of a well-balanced diet, and to prevent constipation by the use of mild laxatives if necessary. Infrequently "obstinate" cases are met with who respond to these measures only after several weeks of rest in bed. Failure of a patient to respond notably after adequate treatment usually means a mistaken diagnosis. Other types of secondary anemia may be benefited by this treatment, but rarely to the same degree as is chlorosis.

The exact mode of action of iron in bringing about

[3] Cabot, R. C., *loc. cit.*

[4] Naegeli, O., *Blutkrankheiten*, etc., 4th Ed., 271.

this improvement is still uncertain. The mere replacement of a deficiency of iron for purposes of hemoglobin formation is not an adequate explanation. It is probable that in this disease iron specifically stimulates the erythropoietic activity of the marrow. Naegeli reports an illustrative case (referred to above) in which after three days of iron administration there was a 27-fold increase in the number of polychromatophilic red cells, with the appearance of some stippled cells, normoblasts, and nuclear particles; a rise in platelets, and a slight neutrophilic leucocytosis, with a few myelocytes—changes which indicate intense marrow activity. He has found regularly a sharp, prompt increase in reticulocytes after iron administration, which in some cases reached high figures. He has observed similar evidence of the stimulating effect of iron on the marrow in occasional cases of secondary anemia, such as that associated with carcinoma.

CHAPTER XIII

PERNICIOUS ANEMIA

PERNICIOUS anemia is a chronic disease of unknown cause characterized by a severe hemolytic anemia, with a high color index; gastrointestinal disturbances with achlorhydria; degenerations in the central nervous system; and a progressive course marked by remissions and exacerbations, with normally a fatal termination.

The term "pernicious anemia" is undoubtedly open to many objections. However, the synonyms which have been used to any appreciable extent (Addison's anemia, Biermer's anemia, "primary anemia") are equally objectionable. The term pernicious anemia, or primary pernicious anemia, is in such general use, and has acquired such a definite significance for physicians generally that it seems best to retain it, at least until an unobjectionable etiological designation can be substituted for it.

Etiology. The cause of pernicious anemia is still undetermined, despite the large amount of experimental work and the even larger amount of hypothesis and speculation which have been devoted to the subject. While it is not yet possible to state what the cause of the disease actually is, it is possible to exclude many hypotheses which have been suggested. In particular, the disease is not caused by hemorrhages, by cancer, by any known poison,

nor by chronic infections, at least of any recognized type. The hypothesis that it is the result of an intestinal intoxication ("auto-intoxication") of some sort is not actually disproved, but it has no convincing evidence to support it.

The constant association of *achlorhydria* with pernicious anemia, and the fact that, as far as observations go, the achlorhydria invariably long precedes the anemia (Cornell [1]), have led to the suggestion that the achlorhydria, directly or indirectly, causes the anemia, possibly by producing an alteration in the bacterial flora of the intestine. However, it seems much more probable that the achlorhydria is at most merely an essential predisposing factor or that, like the anemia, it is a result of the action of the unknown noxious agent, or process, which causes the disease, rather than a directly causative factor in itself.

For a detailed discussion of the more plausible hypotheses which have been advanced to explain the cause of this disease the reader is referred to the critical review of Cornell.

Pathogenesis. The mechanism by which the anemia is produced, the manner in which the unknown noxious agent acts, is also undetermined. Among the main questions at issue are: (1) Are the anemia and the other organic lesions the result of the direct action of some unknown poison? or (2) Are they due to a deficiency of some unknown substance, conceivably a vitamine, which may be supplied by feeding liver?

[1] Cornell, B. S., "The Etiology of Pernicious Anemia," *Medicine*, 1927, vi, 341.

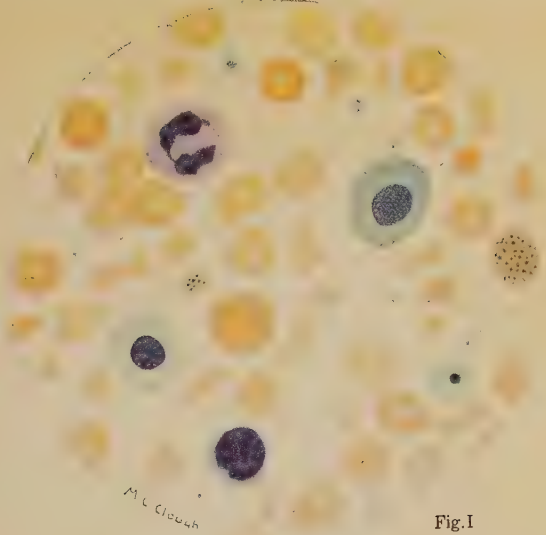


Fig.I

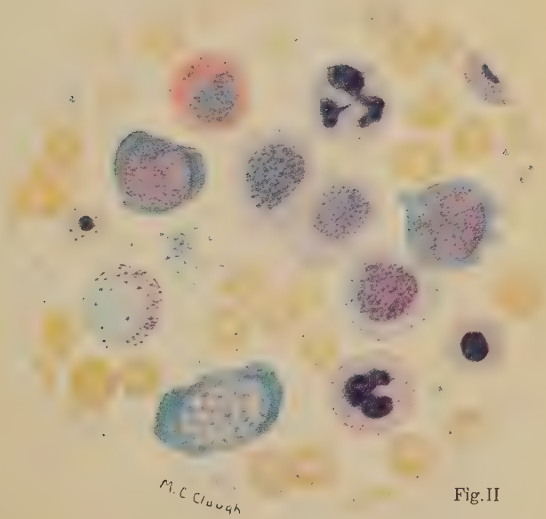


Fig.II

FIG. I. PERNICIOUS ANEMIA
FIG. II. CHRONIC MYELOID LEUKEMIA

There is a great deal of suggestive evidence that some potent toxin is active in the production of the disease. There is no doubt that there is an abnormal degree of red blood cell destruction during the exacerbations of the disease. This is shown, not only by the rapidly increasing anemia, but by the icterus, and by the increase in bile pigments in the serum and urine. At these periods there is an increase in the severity of the other symptoms which is not due merely to lack of hemoglobin, and which may be explained most plausibly as due to the action of some poison. Such symptoms are the fever, the digestive disturbances, the sore mouth, the general weakness and prostration, and the sensory and motor disturbances. The character and wide distribution of the pathological changes in the tissues also suggest the action of some toxin (marrow, nervous system, mucosa of the gastrointestinal tract, myocardium, parenchymatous glands). These degenerative changes cannot reasonably be ascribed directly to a bone marrow insufficiency or to a mere lack of hemoglobin.

Furthermore, blood changes identical with those of pernicious anemia are found in a small proportion of individuals infested with the fish tapeworm, dibothriocephalus latus. Here the blood and marrow injury is almost certainly due directly or indirectly to the action of some poison produced by the presence of the worm, since expulsion of the parasite is followed by complete and permanent recovery from the anemia unless an extremely advanced stage has been reached. It is of great interest that treatment with liver extract has resulted

in prompt and marked improvement of the anemia in such a case, before the parasite was expelled.

On the other hand, there is no direct evidence of the existence of such a toxin.[2] No one ever succeeded in isolating it. Innumerable attempts have been made to reproduce the disease in animals by the administration of a great variety of poisons. It is easy in this way to produce an experimental anemia. With some blood poisons changes have been produced which bear a resemblance to those seen in pernicious anemia in man, but no one has yet succeeded even approximately in reproducing the disease *in toto*, and the blood changes themselves are atypical in important particulars.

It is possible to reconcile the effects of liver feeding with either hypothesis. The prompt and striking changes in the blood after liver feeding indicate unmistakably intense stimulation of the marrow, but the subsidence of those symptoms which depend on injury to other tissues cannot be explained merely by an increase in red cells and hemoglobin, as it is quite out of proportion to the improvement in the blood. The ingestion of liver must supply some substance which not merely stimulates the bone marrow, but which either neutralizes some poison which has been damaging the tissues of the body generally or replaces a deficiency which has resulted in such general tissue injury. It is conceivable that the deficient substance might be a vitamine. It is also

[2] A possible exception to this statement is D. I. Macht's (*Jour. Am. Med. Assn.*, 1927, lxxxix, 759) demonstration that the serum in pernicious anemia exerts a marked inhibitory effect which he believes to be specific, on the growth of lupine seedlings.

possible that it might be some substance produced in the body in normal individuals, but which is lacking in patients with pernicious anemia because of some perversion of metabolism. However, this is still merely speculation. The possibility of maintaining a remission in pernicious anemia for long periods of time, if not indefinitely, by feeding liver, and the apparent necessity of continuing such feeding in order to maintain a remission speak in favor of the deficiency hypothesis.

Another group of workers have suggested that the prime etiological factor consists of some inherent abnormality in the marrow, having the nature of a developmental defect. Thus Piney [3] suggested that there is an inadequate development of the adult (normoblastic) type of marrow, and a compensatory hypertrophy of remnants of the fetal (megaloblastic) marrow which normally disappears completely. The anemia is attributed to the functional insufficiency of this megaloblastic marrow, and extraneous factors, if their existence is conceded at all, are regarded as merely precipitating causes of secondary importance. Adherents of the latter hypothesis emphasize defective cell formation as the factor of prime importance in producing the anemia. The red cells that are formed are believed to be so defective that they speedily fall victims to the (normal) physiological mechanism for the removal of damaged and disintegrating red cells. These writers believe that this is the explanation of the accelerated red blood cell destruction in pernicious anemia, and that it is not necessary to assume an

[3] Piney, A., *Recent Advances in Hematology*, Ed. 2, 1928.

injury to the circulating red cells by any toxin, nor a pernicious overactivity on the part of the reticulo-endothelial tissue in destroying the red cells. Such hypotheses do not give adequate consideration to the other manifestations of the disease. However, the existence of some essential constitutional peculiarity or defect which predisposes an individual to the disease seems quite probable.

Minot also believes that a disturbance of cell formation is of fundamental importance in the production of the anemia. He believes that there is something which retards or prevents the maturation of the erythroblasts (megaloblasts) in the marrow, and that liver acts on the marrow in such a way as to permit, or hasten, this process of maturation.

Pathological Anatomy. The most important and characteristic lesions are:

(1) The bone marrow is hyperplastic, and in the shafts of the long bones, as in the femur, the normal adult fatty marrow is replaced by a soft bright red tissue. Sections or smears from this marrow usually show a predominance of megalocytes and megaloblasts, such as are seen in fetal marrow, in contradistinction to the normoblasts which make up the bulk of the cells of the hyperplastic marrow in the secondary anemias. This feature is usually looked upon as a sign of perverted function, and is frequently referred to as "megaloblastic degeneration."

In the aplastic cases the marrow of the long bones may contain only fat, but similar megaloblastic marrow will usually be found in other bones (ribs,

skull, vertebræ) if a sufficiently extensive search is made.

(2) There is an increase in the iron-containing pigment in the liver, spleen, and lymph glands. In these organs there may be numerous macrophages containing ingested red blood corpuscles.

(3) The central nervous system shows disseminated areas of degeneration, chiefly in the dorsal and lateral columns of the cord (combined sclerosis). The cerebrum and peripheral nerves also show degenerations. The lesions consist of degenerations of the myelin sheaths of the long tracts or nerves without any increase of neuroglia. Lesions may occur without causing appreciable symptoms, and were found in 84 per cent of 96 cases in which they were carefully looked for (Cabot [4]).

(4) Fatty degeneration occurs in the myocardium, and in the glands, especially the liver and kidney.

(5) Atrophy of the lingual mucosa is usually present. Atrophy of the gastric and intestinal mucosa has repeatedly been described, but it is not constant or impressive, and is probably merely a postmortem change. In cases where satisfactory (biopsy) material has been available for examination the gastric mucosa was histologically normal, despite the achylia.

Incidence. The disease is not uncommon, but it is often overlooked. It is a disease of middle and advanced life. It is uncommon under 35, and occurs but rarely in children, at least in typical and definitely recognizable form. However, the possi-

[4] Cabot, R. C., Osler's *Modern Medicine*, Ed. 3, 1927, v, 33.

bility must be kept in mind of encountering in children, and even in infants (Fabian), severe anemias of obscure etiology which respond well to the specific treatment for pernicious anemia. The disease is about twice as common in men as in women.

It not infrequently occurs in two or more members of the same family. Achlorhydria without anemia has been found in a large proportion of the other members of some of these families. This has given support to the suggestion that some inherent individual peculiarity or defect is an essential predisposing factor for the development of the disease.

The disease is about as common in the well-to-do as in the poor, and is as likely to attack robust individuals previously in apparently perfect health as those of poor physique. Draper and others have called attention to certain peculiarities of body type often seen in patients with pernicious anemia. They tend to have fine, prematurely gray hair, a short broad face, and a short deep chest with a wide costal angle. In general they tend to approach the acromegalic type, and males may show some eunuchoid characters, such as an abnormal type of hair distribution and a broad pelvis.

Symptoms. The *onset* is insidious, and a marked degree of anemia usually has developed before a physician is consulted. The common initial symptoms are fatigability, weakness and faintness, headache, dizziness, anorexia or other digestive disturbances, and sometimes palpitation or dyspnea. The patient may have noticed the increasing pallor. Numbness and tingling in the extremities may be early symptoms, and almost invariably appear dur-

ing the course of the disease. These symptoms are highly characteristic and of diagnostic importance. The most constant and usually the most striking symptoms are general weakness and fatigability. Marked as these symptoms usually become, it is surprising how slight they are in the majority of cases, in comparison with the degree of the anemia. In no other condition is one likely to see patients active and relatively free from discomfort with a red cell count under 2 millions. As the disease progresses these symptoms become increasingly pronounced, and all the symptoms arising from a deficiency of hemoglobin appear in more or less marked degree. In extreme cases the weakness may reach such a point that there are periods of unconsciousness or protracted coma.

Of the disturbances referable to the gastrointestinal tract the most important is the *achlorhydria.* This has been reported in such an overwhelming majority of the cases diagnosed as pernicious anemia that it may be regarded as a constant and invariable finding. The few possible exceptions in which the diagnosis of pernicious anemia stands critical inspection do not suffice to invalidate this rule. Simple achlorhydria without anemia, however, is a relatively common occurrence. Probably not over one out of ten such cases ever develop pernicious anemia (Faber). With the achlorhydria in pernicious anemia there is frequently an absence of ferments also, that is, a true achylia gastrica. The achlorhydria in pernicious anemia is permanent. It is not influenced by any form of treatment, and persists throughout the remissions, even when these

are of many years' duration. To demonstrate conclusively the existence of achlorhydria a single aspiration of gastric contents does not suffice. A fractional gastric analysis must be done, on at least two different occasions, as otherwise free hydrochloric acid may escape detection.

The absence of free hydrochloric acid from the stomach juice of otherwise healthy individuals, as a rule, does not give rise to any notable digestive disturbances or discomforts, or to any malnutrition. In those individuals with achlorhydria who do have digestive discomforts it is very questionable whether the symptoms may fairly be attributed directly to the lack of acid. With the exception of diarrhea the symptoms are not relieved with any great regularity by the simple administration of acid. The same is true of patients with pernicious anemia. During remissions digestion is usually normal. During periods of active disease digestive disturbances are usually pronounced. Of these anorexia is the most common. It may be marked and associated with nausea and vomiting, so that taking food becomes almost an impossibility. Common symptoms are epigastric discomfort, fullness and "gas." Diarrhea is common (50 per cent of Cabot's cases),[5] and may be severe, protracted, and very exhausting. There may be constipation, or alternating periods of constipation and diarrhea. Characteristic are abrupt *crises of sharp colicky abdominal pain* that may be so severe as to suggest an impending peritonitis. They come on without adequate external cause, and are refractory to treatment. In many ways they re-

[5] Cabot, R. C., *loc. cit.*

semble the gastric crises of tabes, and it has been suggested that they may be dependent on degenerations in the spinal cord.

Sore mouth is often an early symptom and is a prominent feature at some period of the disease in about half the cases. The process most often involves the tip and margins of the tongue, which looks beefy red and is very tender. Actual ulceration is uncommon. In some cases the mucous membrane of the mouth and throat is extensively involved, and there may be marked burning and pain on swallowing. The administration of acid is torture to such patients. The discomfort often seems out of proportion to the visible lesions in the mouth. The process is practically uninfluenced by any form of local treatment, but subsides spontaneously when a remission occurs.

The most common symptoms referable to the nervous system are the *paresthesias* in the extremities. They are almost invariable, and in a majority of the cases of pernicious anemia they are the only subjective symptoms referable to the nervous system. There may be no demonstrable lesions in the cord to explain them, and it is probable that they depend on lesions of the peripheral nerves. Objective loss of sensation is uncommon in these cases except diminution or loss of the vibratory sense, especially over the tibia.

Cerebral disturbances are common, especially headache, irritability, lack of power of concentration, mental dullness or drowsiness, or more rarely delirium. Coma frequently supervenes before death.

Pronounced symptoms due to *focal degenerations*

in the spinal cord appear in a smaller but still considerable number of cases. These consist of (1) spasticity of the muscles, chiefly of the legs, with weakness or paralysis, exaggerated reflexes, and a positive Babinski phenomenon, dependent on degenerations in the lateral columns of the cord; or (2) ataxia, with weakness, diminished reflexes, and more or less loss of sensation, dependent on degenerations in the posterior columns,—a symptom complex resembling tabes. In most of these cases careful examination will show evidence of involvement of both columns, with a more or less mixed symptom complex. In Cabot's series 46 cases were predominently of the spastic type and 75 cases of the ataxic type.[6] In the later stages the "spastic" cases usually develop some anesthesia and sphincter disturbances.

These cord degenerations may develop at any stage of the disease. There is a well-defined group of cases in which they precede the anemia or in which the blood changes at first are slight. There is also a group of cases (so called "pure" combined sclerosis, also associated with achylia), in which the disease runs its course to a fatal termination without the development of anemia. The relation of these cases to pernicious anemia is not yet definitely determined, but many regard them as pernicious anemia without anemia.

Once established, these degenerations are incurable. With remissions, especially as a result of liver feeding, the progress of the lesions is checked. With improvement in general strength, and perhaps partly

[6] Cabot, R. C., *loc. cit.*

as a result of training and reëducation there may be symptomatic improvement and increasing ability to use the muscles. Nothing approaching complete recovery is to be expected as a rule, although in rare instances the degree of improvement has been surprisingly marked.

Slight degrees of cord degeneration undoubtedly occur more frequently than clinical statistics indicate. A thorough neurological examination should be made in every case of suspected pernicious anemia, with special reference to the condition of the reflexes, to the presence of ataxia, and of minor sensory disturbances. Abnormalities are found in many cases without symptoms which would suggest the presence of such lesions.

Physical Examination. The *color* of the patient is the most striking feature on physical examination. Most often there is merely a marked pallor. During the exacerbations of the disease, while there is increased blood destruction, there is a distinct pale yellowish tinge that may deepen to a definite jaundice. There is often some brownish pigmentation of the skin, independent of jaundice. Petechiæ may occur, but are rare except in the retina.

Nutrition is relatively well preserved. There is usually some loss of weight. This is often marked, but is rarely extreme, and the patients do not look cachectic, as they so often do with cancer.

The *tongue* is abnormally clean. It usually has a dry smooth polished appearance due to atrophy of the papillæ, which is often complete along the margins and at the tip. This is a nearly constant and highly characteristic phenomenon, but it is not

pathognomonic since some degree of atrophy may be seen in other conditions associated with achlorhydria. If an active stomatitis is present the tongue may be injected. Oral sepsis and pyorrhea are common, but are probably only incidental findings. A glossitis of the type described by Hunter is a rare phenomenon in the experience of the writer.

The *heart* is usually a little dilated, occasionally quite markedly so. Systolic murmurs over the whole precordium are almost invariable, and they may be intense. The sounds are not altered, as a rule, and diastolic murmurs are rare. The blood pressure is rather low. The spleen is usually a little enlarged, but only occasionally palpable. The liver is frequently a little enlarged and hard.

Blood. The changes in the blood vary in detail, depending on the rate of formation of new red blood cells and the degree to which the function of the marrow is perverted. In the average case there is a marked reduction in the total red cell count, often to 2 millions or less when the patient first comes under observation. The hemoglobin is also greatly reduced, but to a less degree, so that the color index is high, 1.0 to 1.5 or more. The drop of blood as it emerges looks pale and watery, and is much less viscid than normal. Microscopic examination shows fairly numerous *poikilocytes*. Even more striking is the *anisocytosis*. Not only are many individual cells far below, and far above, the normal size (diameter), but among the others there is such a marked variation that one gets the impression of a heterogeneous lot of cells, no two

of which are absolutely identical. The average size of the individual cells is above normal, and their color is intense. These impressions are confirmed by actual measurements of the cells. If the diameter of 1,000 red cells is measured with a micrometer, the mean diameter in pernicious anemia is found to be about 8.6 microns, as compared with the normal of 7.5, and if these individual measurements are plotted graphically by the Price-Jones method, or in some similar way,[7] it will be found that the dispersion of the measurements (which is a measure of the anisocytosis) will be about double that of normal blood.

One of the most characteristic features of the blood in pernicious anemia is the presence of numerous large oval, deeply staining cells (macrocytes). As Duke has pointed out, there is a decided tendency for these large cells to accumulate in the peripheral capillaries. As a result the red cell count and hemoglobin content of capillary (finger) blood may be substantially higher (20 to 30 per cent) than that of venous blood, and thus give an inadequate idea of the actual severity of the anemia. With return of the blood to normal during a remission this discrepancy disappears (Plate I).

The volume index is increased above 1.0, more markedly and more regularly than is the color index. The cells are thick as well as broad. This increased thickness of the cells is the reason for their dark color. The concentration of hemoglobin

[7] Bell, J. R., Thomas F. K., and Means, J. H., *Jour. Clin. Investigation*, 1926, iii, 229. Medearis, D. N., and Minot, G. R., *Ibid.*, 541.

in the cell substance is not increased, the saturation index is normal, or somewhat below normal.

The *platelets* are much reduced in number. "Giant" platelets are common, and rarely a megakaryocyte, or a fragment of one may be found (Plate II). The *leucocytes* are also diminished, and the proportion of granular cells is low. A few myelocytes are not uncommon. It is common to find a few giant leucocytes, huge neutrophiles with nuclei containing an unusually large number of segments (Plate II). The leucocytic index (Arneth) is shifted to the right.

With the onset of a remission, or during periods of active cell regeneration, there also appear in the blood many immature red cells, reticulocytes, polychromatophilic and stippled red cells, and nucleated red cells or cells with nuclear particles. The *nucleated red cells* may be normoblasts alone, or megaloblasts, or both. The number of nucleated red cells present, as a rule, is small. Except during periods of marked marrow activity they are usually decidedly sparse, and protracted search may be required to find one. Failure to find them on a single examination is common, and by no means speaks against the diagnosis. They can be found almost invariably at some period of the disease if repeated careful examinations are made. By a careful examination is meant a systematic survey of several films with the aid of a mechanical stage, requiring from 3 to 6 hours' time.

Normoblasts are found more commonly than megaloblasts, and are usually found in larger numbers. The finding of megaloblasts is emphasized

because of their greater diagnostic significance. This significance depends largely on how the term megaloblast is defined. If one classes as a megaloblast any large red cell with a nucleus which is not pycnotic, as is rather commonly done, they have relatively little significance, as such cells are found not uncommonly in a variety of other anemias. If, however, one restricts the use of the term, as does Naegeli, to those large cells (megalocytes) which in their primitive state possess a nucleus with a relatively fine reticular structure, their significance is greatly increased, as cells of the latter type are rarely found in conditions other than pernicious anemia (see page 16). On the other hand the finding of a few mature or immature normoblasts is of no real aid in differential diagnosis.

Normoblasts occur only in the presence of definite anemia. Megaloblasts occur in appreciable numbers only if anemia is pronounced. However, an occasional megaloblast may appear in the absence of notable anemia, as in the initial stages of the disease or during a spontaneous remission. At such times they are extremely scarce, and attempts to find them are often unsuccessful.

There occasionally occurs an outpouring into the blood stream of nucleated red cells, both normoblasts and megaloblasts, in considerable numbers, a so-called *blood crisis*. This is associated with other evidences of marrow stimulation, including a rise in the leucocyte count, and the appearance of a few myelocytes. Such a crisis sometimes initiates or accompanies a period of remission, but more often it seems to be the expression of a spasmodic

and futile attempt on the part of the marrow to cope with the anemia, and the downward course of the disease continues without interruption. Contrary to the impression left by many textbooks, these blood crises are relatively uncommon in pernicious anemia, and usually have an unfavorable prognostic significance.

During the periods of rapid blood destruction the serum is yellow from the presence of bilirubin. It gives a positive indirect Van den Bergh reaction and shows a high icterus index. The urine is dark colored from its high pigment content. In the intervals the urine is pale. There is usually a moderate degree of albuminuria and cylindruria.

To *summarize*, the blood changes which are of chief diagnostic significance are: the severity of the anemia, the high color index and high volume index, the dark color and large average size of the individual red cells, the marked degree of poikilocytosis, and especially of anisocytosis, the presence of many large oval, deeply staining red cells, and (often only after repeated and protracted search) the finding of typical megaloblasts. Failure to find nucleated red cells on a single examination is of relatively little significance. The presence of large numbers of normoblasts in general is a point against the diagnosis of pernicious anemia. Abundant platelets or a leucocytosis speaks strongly against the diagnosis of pernicious anemia.

Course of the Disease. The *remissions* constitute a highly characteristic feature of the disease, and occur in a large majority of the cases. The effect of the administration of liver on the course of the

disease will be considered in a later section. In patients otherwise treated, after a period of weeks, or usually months, of progressive increase in the weakness, anemia, and other symptoms, there comes an arrest in the progress of the disease and an improvement in all the symptoms except those due to degeneration of nerve tissue. Remissions may occur in patients who have reached extreme degrees of anemia and debilitation, or who are even in coma. A striking feature of the remissions is their occurrence quite irrespective of the method of treatment that may have been employed (other than liver). Remissions may undoubtedly occur spontaneously in patients receiving no specific therapy. Naegeli insists, however, that they occur far more frequently since arsenic therapy came into general use (about 1877), and that prior to this spontaneous remissions were comparatively rare.

Remissions may begin with considerable abruptness and symptomatic recovery may proceed rapidly. Relief from the distressing symptoms and a feeling of relative well-being are the first evidences of improvement, and they precede and are out of proportion to the increase in red blood cells and hemoglobin. As pointed out by Cabot[8] and others, there is not an exact parallelism between the intensity of the symptoms and the degree of the anemia. The symptoms vary more nearly with the rate at which the disease process is progressing.

The degree of recovery during a spontaneous remission varies greatly. Often it is relatively slight. In most cases there is substantial improvement,

[8] Cabot, R. C., Osler's *Modern Medicine*, Ed. 3, 1927, v, 33.

but still considerably less than complete restoration to normal. In a smaller number there is complete symptomatic recovery, so that the patient returns to his work and regards himself as cured. In such cases the blood also approaches normal, but rarely quite attains it. Although the hemoglobin may reach normal, noticeable qualitative changes in the red cells persist. The color index remains a little high, the cells are dark, some anisocytosis persists, with occasional oval dark-colored macrocytes, and the average size of the cells is still above normal. Rarely an isolated megaloblast may be found.

The duration of the remissions is also variable. It is usually under a year, and may be a few weeks only. Remissions of more than one year are not rare (about 4 per cent in Cabot's series) and they may last from 4 to 6 years. Sooner or later a relapse occurs, with reappearance of all the symptoms, usually in more marked degree than during the first attack. Red blood cell destruction is accelerated, and there is a progressive increase in the anemia. A fair proportion of these cases may have a second remission, and rarely there may be as many as five remissions. In Cabot's series, about 20 per cent showed no distinct remission, 80 per cent had one or more, 30 per cent had two or more, and only 15 per cent had more than two. Subsequent remissions are usually less complete and of briefer duration than preceding ones, and each exacerbation is more severe, until death finally occurs, usually as a direct result of the disease itself, rarely of an intercurrent infec-

tion. If spontaneous recovery ever occurs it is so rare as to be of no practical significance.

Distinct remissions do not occur in all cases. There is a group of cases in which the disease runs a more acute course without interruption, with death within six months. There is also a group of cases which progress more slowly, and after a considerable degree of anemia has been reached may remain in a practically stationary condition for weeks or months, after which the disease again progresses, to a fatal termination. Such cases are apt to show an aplastic blood picture, with little or no evidence of active cell regeneration.

The duration of the disease from the onset of outspoken symptoms in most cases is from one to three years. Occasional cases live much longer. Cabot [9] reported that 20 per cent of his series lived over 3 years. He collected 37 long cases, living over 7 years, 3 of them from 14 to 15 years.[10]

Diagnosis. After the disease has advanced to the stage in which there is obvious anemia its recognition rarely offers any real difficulty. It is essential not only to examine the blood, but to take a careful history, make a thorough physical examination, and examine the gastric juice. Neglect of any one of these measures may lead to error. The blood examination, of course, is of prime importance, and the characteristic changes will rarely escape the practiced eye. Even in the early preanemic stages they are sufficient to arouse suspicion, and after

[9] Cabot, R. C., *loc. cit.*

[10] The entire discussion in this section is applicable only to patients who have not received liver therapy.

anemia has developed they are so highly characteristic that in most cases they alone suffice to establish the diagnosis with a high degree of probability.

However, there is abundant evidence that anemia is a late manifestation of the disease, and that in many cases, at least, milder but characteristic symptoms may precede the appearance of the anemia, even by some years. These symptoms vary somewhat in individual cases, but the most common and characteristic are sore mouth with aphthous ulcers, vague digestive discomforts, diarrhea, and numbness in the finger tips of both hands. Somewhat later there may be neuralgic or neuritic pains, and a clean polished looking tongue with beginning atrophy of the papillæ. Thorough examination even in these early stages will invariably show an achlorhydria, and usually incipient changes in the blood; without a perceptible anemia, there may be a somewhat high color index with a macrocytosis and some typical large oval dark cells. These changes are not marked and will probably be overlooked unless the possibility of pernicious anemia has been suggested by the history. The writer regards the typical changes in the tongue, achlorhydria, and paresthesias in the extremities as a triad of early symptoms which are so characteristic as to be practically pathognomonic of pernicious anemia. There is good reason to believe that early recognition and treatment of these cases will avoid much unnecessary invalidism and suffering, and will prevent the development of incurable degenerations of nervous tissue.

Differential Diagnosis. (1) Diseases which give rise to a *secondary microcytic anemia*, such as cancer, intestinal parasites, certain chronic infections, and chronic hemorrhage, rarely present real difficulty in diagnosis. An adequate blood examination alone will exclude pernicious anemia in nearly all of these cases. There may be real difficulty in cases of pernicious anemia associated with hemorrhage. The color index may then be low and the cells pale, but the other characteristic features of the disease are not altered.

(2) Diseases associated with a *megalocytic anemia* are more difficult to differentiate. Among these may be mentioned:

Dibothriocephalus latus (fish tapeworm) *infestation* in rare cases causes an anemia with blood changes which are identical with those of pernicious anemia. Examination of the feces for ova or segments of the worm suffices to make the diagnosis. Permanent cure follows prompt expulsion of the worm. The parasite is common in the Scandinavian countries. Certain fresh water fish in some districts in the Great Lakes region in this country have become infested, and several indigenous cases of anemia have been reported in inhabitants of the large cities who had eaten uncooked smoked fish shipped from these regions. Naegeli mentions two similar cases of anemia associated with Tenia saginata infestation.

Pregnancy. In relatively rare cases a hemolytic type of anemia which resembles pernicious anemia may arise during pregnancy or the puerperium. This type of anemia in the severer cases runs a more

rapid course than does typical pernicious anemia, without remissions. With termination of the pregnancy artificially or spontaneously many of the cases recover completely and permanently. Transfusions are of great value, according to Larrabee. Some patients have gone through subsequent pregnancies without difficulty. This anemia occurs chiefly in women under 35, an age at which typical pernicious anemia is rare. Pregnancy is practically unknown in women with the classical relapsing form of pernicious anemia. It seems probable that the anemia of pregnancy is a distinct disease, with a more favorable prognosis.

Acute aplastic anemia may resemble some of the more acute cases of pernicious anemia. It differs clinically in that it occurs in younger individuals, runs a rapid course without remissions, and shows no evidences of rapid hemolysis or of active cell regeneration.

Cancer may very rarely cause a megalocytic type of anemia, chiefly in cases involving the stomach or intestine, or in cases with metastases to the bone marrow. The blood changes are not perfectly typical (see page 113). In the few recorded cases of typical pernicious anemia in which an unsuspected gastric cancer was found at autopsy there may well have been an accidental coincidence of the two diseases.

Sprue in certain cases presents perhaps greater difficulty in differential diagnosis than does any other condition. Common to both diseases are digestive discomforts, diarrhea, sore mouth, paresthesias of the extremities, and an anemia of the

macrocytic type. Some authorities claim that the two diseases are identical. The writer cannot agree with this view, although they are probably closely related conditions. It is interesting to note that several cases have been reported which were benefited by liver feeding. The majority of the cases of sprue can be differentiated from pernicious anemia without undue difficulty. In sprue there is often marked emaciation, rare in pernicious anemia. There is persistent morning diarrhea with bulky, frothy stools. In the majority of the cases of sprue there is free hydrochloric acid in the gastric juice, and the tongue does not usually show the same degree of papillary atrophy. The blood changes differ in detail; they are less marked, macrocytosis is distinctly less prominent, and megaloblasts rarely, if ever, occur.

Syphilis and *malaria* are referred to repeatedly in the literature as possible causes of a megalocytic anemia. The number of reported cases that deserve any consideration is so small that they may be merely coincidences. Naegeli, however, has reported one patient with active syphilis who was permanently cured of a megalocytic anemia by salvarsan and mercury.

Severe anemias in children, particularly some of the cases classed as anemia pseudoleucemica infantum, may bear some resemblance to pernicious anemia. These cases differ in that they show a leucocytosis, abundant platelets, and large numbers of nucleated red blood cells, and they have none of the clinical symptoms except anemia. Typical pernicious anemia is rare in children, and this diagnosis

should be made in children only after most careful consideration.

Leucemia may offer difficulty in rare cases (see Leucanemia).

Treatment. The administration of liver in adequate quantities, or of suitable liver extracts, as first advocated by Minot and Murphy,[11] has given results so much superior to those obtained by previous methods of treatment that at present it deserves primary consideration. Other measures are important but must be regarded as subsidiary.

The general hygienic and dietary measures which would be indicated in any chronic debilitating disease should be instituted in pernicious anemia. Rest in bed for the first few weeks is important, preferably in the open air and sunshine. Focal infections should be eliminated as far as possible, particularly periapical tooth abscesses. While there is no convincing evidence that such infections are a direct cause of the disease, as some have maintained, there is no doubt that they exert a deleterious effect on the general health.

The *diet* should be liberal and well balanced. Minot and Murphy advise that it include 400 grams of fruit per day, preferably fresh, 300 grams of green vegetables, and 100 grams of red meat in addition to liver. They at first emphasized the restriction of fats, including milk products, on the theoretical ground that the disease may be due to some perversion of fat metabolism. Experience has shown that the inclusion of fat does not lessen the

[11] Minot, G. R., and Murphy, W. P., *Jour. Am. Med. Assn.*, 1927, lxxxix, 759.

effectiveness of liver, and there is no definite evidence that fat in itself is injurious. Moderate quantities of cereals, bread, potatoes and simple desserts may be taken if desired.

According to Minot and Murphy, the minimum effective quantity of *liver* is about 120 grams per day. The average patient requires 200 grams per day, and some patients who fail to respond to this quantity will improve if double this amount is taken. The source of the liver (mammalian or bird) and the mode of preparation seem to be immaterial except as palatability and digestibility are concerned. Raw liver is fully as effective as cooked liver.

When treatment is started the feeding of liver, or of any food whatever, is often difficult on account of the anorexia and digestive disturbances, and only a small fraction of the optimum daily dose can be eaten. An effort should be made to increase the quantity gradually, until a minimum of 100 grams a day is reached by the end of the first week. If this can be accomplished the appetite usually improves and increasing quantities can be eaten, until the full quota is reached. In many cases the patients acquire a craving for liver, and may eat it ravenously. The capacity of consuming with relish such large quantities of liver over long periods of time seems almost a specific characteristic of the disease, and would be quite impossible for most normal individuals.

In many cases the initial distaste merely lessens, and persistent effort is required to keep up the diet. The marked subjective improvement enjoyed by

the patient as a result is usually a sufficient incentive to persist. For patients who have only moderate difficulty in eating the liver a good cook is of considerable assistance in preparing the dishes attractively and in varied form. If the distaste is great such measures are of little avail, and the most practicable method of administration may be in the form of a suspension of finely ground raw liver in orange juice. Such a mixture can often be drunk by the patient when mastication of cooked liver is impossible. It is usually preferable to use liver extracts in such cases. This obviates any possibility of parasitic infestation from the raw liver.

In patients with extreme anorexia, or in unconscious patients, such suspensions may be given by stomach tube. In such cases the writer strongly favors giving promptly one or more transfusions in conjunction with liver extract, as they often lessen digestive disturbances and facilitate taking the liver.

The first *effect of an adequate quantity of liver* is a rise in the percentage of reticulated red cells. This begins within a few days (usually 3), reaches a figure of about 10 per cent after about a week, and then falls as rapidly again to normal. With the rise in reticulocytes symptomatic improvement usually begins. The appetite improves, digestive disturbances lessen, strength increases and fever if present disappears. The yellow color of the skin, the bilirubin in the serum, and other signs of increased blood destruction diminish. A rise in red cells, and to a less extent in hemoglobin, next becomes manifest. In Minot and Murphy's early

cases the average rise in red cells in the severer cases was from 1.5 to 3.3 millions in one month, and to 4.2 millions in two months. In general the more satisfactorily the diet is taken, the more rapid is the rise in the count and the more nearly normal the blood becomes. When the liver is taken persistently and satisfactorily the red cell count remains above 4.5 millions per c.mm. In occasional cases the count may become too high and the amount of liver eaten must be reduced. With the restoration of a normal red cell count, the color index and the volume index fall to 1.0 or below, and the qualitative changes in the red cells disappear, in particular the anisocytosis and macrocytosis (the Price-Jones curve becomes normal), so that it may be impossible to tell from the blood film that pernicious anemia has ever existed. In this respect the remissions induced by treatment are more complete than the most favorable spontaneous remissions, as in the latter some qualitative abnormalities in the red cells almost invariably persist. The granular leucocytes and platelets also increase to normal.

Patients without notable anemia are also benefited in that the progress of the disease is arrested. Actual degenerations of nerve tissue are not repaired and abnormalities in reflexes persist. In some cases, however, considerable symptomatic improvement in the nervous symptoms occurs. The achlorhydria persists and usually the lingual atrophy.

The liver diet has received extended trial in numerous clinics throughout the country, and the

results in general fully confirm the claims of Minot and Murphy. A few refractory cases are recorded. Minot and Murphy state that "there may be a rare case of this disease that cannot respond to liver alone," but that "essentially all patients with pernicious anemia can be benefited, and usually markedly and promptly." They regard a failure to secure improvement after 6 weeks' treatment as nearly always indicating either a mistaken diagnosis or an insufficient amount of liver. Failure may be due to intercurrent infections or associated chronic diseases. Inevitably there is much individual variation in response to treatment. Not all patients will show satisfactory improvement under the quantities of liver which it is possible to induce them to eat, and which suffice in most cases. This is not surprising, but rather the comparative rarity of such cases.

The exact nature of the active substance in liver has not been determined. Cohn, in collaboration with Minot *et al.*,[12] has concentrated the active substance, practically without loss, in therapeutically effective *extracts* of which a few tenths of a gram represent 100 grams of fresh liver. These extracts are free from proteins, from fats and fat soluble lipoids, and from iron. The effectiveness of liver in pernicious anemia, therefore, can not be due to proteins or other nutritive substances it contains, nor to the fat soluble vitamine A, as was maintained by Koessler. The active substance, which is readily soluble in water, must be something present in liver in small concentration, since as little as 0.6

[12] Cohn, E. J., *et al.*, *Jour. Biol. Chem.*, 1928, lxxvii, 325.

grams per day of a concentrated extract has sufficed to stimulate the production of reticulocytes in pernicious anemia. Minot believes it is probably a relatively simple nitrogenous base.

Satisfactory extracts (less concentrated) have been prepared on a commercial basis (Liver extract No. 343, N. N. R.) and are desirable for patients who have difficulty in eating liver. The usual initial daily dose is 5 or 6 vials (the extract from 500 or 600 grams of liver). This may be reduced to 3 or 4 vials a day after improvement is well under way, while 2 vials a day may be a sufficient maintenance dose. Active extracts in these doses are fully as effective as whole liver in pernicious anemia, and are easily administered in solution in fruit juice. However, some preparations of extract have been deficient in activity, and as there is no method of standardization except by trial on patients, whole liver should be substituted or added whenever a patient fails to respond satisfactorily to a commercial extract. The extract is not a satisfactory substitute for whole liver in secondary anemias.

Several important questions naturally arise concerning the end results of this treatment.

(1) How long do these remissions last?

(2) How long must liver be continued?

(3) Does this treatment cure the disease?

The answers to these questions are now fairly clear. It is certain that individual patients have been kept well for 5 years and more by liver feeding and that practically all patients who have continued to take adequate quantities of liver have remained well for periods of from one to 5 years.

It is certain that in most cases, at least, liver must be kept up indefinitely. The quantity required to maintain a remission in favorable cases is substantially less than that required during the active phase of the disease. In unfavorable cases a remission may be maintained only by persistent administration of large quantities of liver. If liver is stopped or too much reduced a relapse occurs, sooner or later. These relapses respond favorably to increased quantities of liver. The maintenance dose must be determined by trial for each individual case. It is probable that 300 grams a week represents the minimum requirement and that in many cases substantially larger amounts are necessary.

These facts would indicate that a radical "cure" has not been achieved. Liver probably produces merely an arrest of the disease usually amounting to a symptomatic cure, which can generally be maintained for years, and perhaps indefinitely, by continued treatment.

While improvement under treatment with liver is so nearly constant in pernicious anemia that it seems to be a specific characteristic of the disease, the converse is not true. Some patients with other types of anemia may also be benefited, although not with the same regularity, nor usually to the same degree. Improvement under liver therapy, therefore, should not be regarded as proof that a condition so benefited is necessarily pernicious anemia.

Drug Therapy. *Iron* is useless. There is a surplus of iron in the tissues. The anemia is not due to a lack of iron, nor primarily to a lack of hemoglobin, but to a lessened ability of the tissues to

form red cells. Clinical experience has shown that iron does not effectively stimulate this function in pernicious anemia.

Arsenic deserves somewhat more serious consideration. The clinical experience of the past seems to indicate that it is of some benefit, probably by acting as a stimulant to the erythropoietic tissue. There is no doubt that in large doses it acts directly as a poison to this tissue. There is no conclusive evidence of its therapeutic effectiveness, and at best this is insignificant as compared with that of liver. With present methods of treatment arsenic is superfluous, and probably is more likely to injure the patient by disturbing the appetite and digestion than to help him. Arsenic preparations administered hypodermically or intravenously are no more effective than if given by mouth, but are somewhat less likely to upset digestion. The method of choice, if arsenic is to be given at all, is by hypodermic injections of sodium cacodylate (1 grain a day, in courses of ten injections, with an interval of 7 to 10 days between courses). Fowler's solution may be given by mouth if it does not upset digestion. Naegeli favors arsacetin and is a strong proponent of its effectiveness.

..*Hydrochloric acid* has been used extensively, more because of the presence of an achlorhydria than because of any convincing evidence that it is really beneficial. The writer is sceptical as to its efficacy, either as a curative or symptomatic remedy, except that it may sometimes lessen diarrhea. However, it should usually be tried except in patients who

suffer discomfort after taking it. Relatively large doses are desirable: 2 cc. of the dilute acid during or immediately after the meal, and repeated twice at half-hour intervals after the meal.

Transfusions properly administered have been an important aid in treatment, and are still valuable in certain cases. Past experience has shown conclusively that transfusions do not cure the disease. There is no conclusive evidence that they will bring about a remission, nor that on the average they have prolonged life, although there can be no doubt that they have done so in certain individual cases. Transfusions have been especially valuable in relieving discomfort and increasing the strength of the patients. They help to relieve all those symptoms which are due directly to a lack of hemoglobin, and in particular they often relieve anorexia and other digestive disturbances and improve the appetite. As a result of this effect the writer is convinced that in occasional cases at least, the patient has been supported through a period of depression and life has been prolonged until a remission was initiated.

Transfusions should be given promptly in most cases when the red cell count is under 1 million, and not infrequently they may be indicated in the presence of a higher count if the patient is greatly debilitated or has marked anorexia. The successful results of liver therapy have greatly restricted the field of usefulness of transfusions.

Splenectomy is now of historical interest only. It is probable, however, that before liver treatment

was introduced, in occasional cases splenectomy brought about a remission after other methods of treatment had failed. Such a remission was usually the last one. The postoperative mortality probably offset such an occasional temporary benefit.

CHAPTER XIV

SICKLE CELL ANEMIA

THIS is a chronic familial disease of unknown etiology, limited to negroes, and characterized by the tendency of the red cells to assume characteristic bizarre shapes (sickle cells, stellate cells), and in the outspoken cases by recurring periods of rapid destruction of red blood cells which result in the development of a severe anemia of a hemolytic type.

Symptoms. In the cases showing outspoken symptoms the disease runs a chronic course, marked by remissions and exacerbations. The chief symptoms during an exacerbation are (1) those due to anemia—weakness, fatigability, dyspnea, palpitation; (2) digestive disturbances—pain in the epigastrium or region of the spleen, with nausea and vomiting, recalling the crises seen in pernicious anemia, and apparently directly associated with rapid blood destruction; (3) pains and stiffness in the joints and muscles, often severe and incapacitating; and (4) in the later stages chronic leg ulcers superficially resembling syphilitic ulcers. During the more acute stages there is moderate fever, prostration, general malaise, slight jaundice, and all the other symptoms and signs of rapid destruction of red blood cells. Examination shows the patient to be undernourished, and development

to be retarded. The skin and mucous membranes show pallor and slight icterus. The liver is somewhat enlarged. The spleen is usually small and fibrotic, but in young individuals it may be palpable.

The *blood* in severe cases shows a marked reduction in hemoglobin (30 to 60 per cent), and in the red cell count (1 to 3 millions), with a color index which is usually slightly below 1.0. A considerable degree of anisocytosis is present, with many microcytes. The average color of the red cells is about normal. There are occasional polychromatophilic and stippled red cells, and often a few normoblasts. There is usually an increase in platelets and a neutrophilic leucocytosis of 10,000 to 20,000 with an occasional myelocyte.

The fundamental characteristic of the disease is the tendency of the red cells to develop these peculiar deformities. This is present at birth and persists throughout life. In stained films only a few of the red cells show these deformities, and these few can be found only in patients with outspoken anemia. They can be demonstrated satisfactorily only in fresh preparations which are sealed with petrolatum to prevent evaporation and incubated for some hours. The characteristic change consists in the apparent drawing out of the cytoplasm of the red cell at two or more points into elongated spine-like projections. This gives to some of the cells the sickle shape to which the disease owes its name, but an even larger number become oat-shaped or irregularly stellate. At the end of 24 hours practically all the cells will have assumed this shape.

The cells so distorted are abnormally susceptible to phagocytosis in warm stage preparations. Increased phagocytosis also occurs in vivo, as shown by a study of the tissues at autopsy, and by the occurrence in blood films of occasional macrophages containing ingested red blood cells.

Course. After a few weeks of acute illness there will be an amelioration of the symptoms, and the patient will enjoy a few weeks or months of comparatively good health. In the severe cases this improvement never approaches complete recovery, and there is permanent disability and invalidism of varying degree. In such cases the disease tends to increase in severity, and death may occur from anemia or from intercurrent infection. The outlook is bad in children with severe symptoms. The prognosis as to life is more favorable in patients who survive to become adults.

The severe form of the disease occurs in but a small proportion of affected individuals. It has been shown, particularly by Sydenstricker,[1] that the blood from healthy relatives of patients with this disease in many cases will show typical sickling. There is every gradation from individuals with no symptoms whatever, to the severest cases of the disease. The condition in such symptomless cases is commonly known as "*latent sickle cell anemia.*" The latent cases much outnumber the patients with actual anemia.

The change in shape of the cells is certainly due to a peculiarity or defect of the cell itself, and not

[1] Sydenstricker, V. P., *Jour. Am. Med. Assn.*, 1924, lxxxiii, 12.

to any substance in the serum. According to Hahn and Gillespie [2] the change in shape occurs when susceptible cells are deprived of oxygen, and a reversal to the normal circular outline can be brought about by oxygenating the preparation containing sickled cells. There is a direct relation between the acuteness of the disease process and the number of sickled cells in freshly made films, but even in the symptomless cases without anemia practically all the cells eventually will sickle in incubated preparations. It is probable that the increased phagocytosis and accelerated blood destruction are due to defects in, or injuries to, the red cells, rather than to a primary overactivity of the macrophages. However, the resistance of the red cells to hypotonic salt solution is not diminished—an important point in the differentiation from familial hemolytic jaundice.

Differential diagnosis. The clinical picture of the disease presents points of similarity to both pernicious anemia and familial hemolytic jaundice. Examination of the fresh blood is essential for a correct diagnosis and establishes the latter with certainty, since the changes in the red cells are unmistakable and pathognomonic.

Treatment. The disease is incurable, and treatment is purely symptomatic. Iron is probably useless. No notable effect has yet been reported from liver therapy. In the severe cases transfusions are the most effective measure to relieve the symptoms

[2] Hahn, E. V., and Gillespie, E. B., *Arch. Int. Med.*, 1927, xxxix, 233.

of anemia. Splenectomy has been tried in a few cases, with alleged improvement in one or two while others were unimproved. The procedure is still in the experimental stage, and improvement from it, if any, is likely to be only temporary.

CHAPTER XV

HEMOLYTIC JAUNDICE

HEMOLYTIC jaundice is a chronic disease, or rather a group of diseases, characterized by recurring periods of abnormal red blood cell destruction, with the development of an acute hemolytic anemia and jaundice, by enlargement of the spleen, and by a diminished resistance of the red blood cells to the hemolytic action of hypotonic salt solution.

Two types of the disease occur, the congenital, familial form (Chauffard-Minkowski type), and the acquired form (Hayem-Widal type) in which there is no demonstrable familial tendency.

Familial Hemolytic Jaundice is the commoner form of the disease, and constitutes a clear-cut clinical entity. It depends on a hereditary constitutional anomaly, and no other etiological factor has been demonstrated. The anomaly is present from birth, although notable symptoms may not appear until a few years later. The disease affects males and females equally, and it is transmitted by either parent as a dominant Mendelian characteristic, so that an average of only half the children in a family are affected. The disease is not transmitted by a healthy individual. However, the possibility of latent cases is suggested by the fact that typical con-

genital cases occur, without a history of the disease in either parents or siblings.

Symptoms of the disease may be manifest from birth, but more often they appear later, during the first or second decade. Their first appearance may be precipitated by some acute infection. In a majority of cases the symptoms are mild, and during the greater part of the time the patients feel well, or complain only of slight weakness and fatigability, and of a sallow or yellowish discoloration of the skin. At irregular intervals of usually three to four months an exacerbation of symptoms occurs, associated with a period of accelerated blood destruction, a "crisis of deglobulization." There is a considerable degree of malaise and disability. In the severer cases there may be fever, prostration, anorexia, vomiting, and (even in the absence of gallstones) attacks of sharp abdominal pain referred to the epigastrium or left hypochondrium. Outspoken jaundice appears or the pre-existing color deepens. The face often shows a brownish yellow or buff color, and in some cases becomes deep orange, but does not show the green or bronze tint seen in some cases of chronic obstructive jaundice. All the other phenomena characteristic of an acute hemolytic anemia appear in the most marked degree. The serum becomes heavily bile stained, has a high icterus index, and gives a strongly positive indirect Van den Bergh reaction. The quantity of bilirubin may be increased to 10, 20, or even 50 times the normal. The urine becomes dark, from increase in pigments (but not bilirubin), and the quantity of bile pigments in the feces is greatly in-

creased. This excessive excretion of bile favors the formation of gallstones, which are present in over half of the cases (Mayo). The symptoms and signs of cholelithiasis and cholecystitis are, therefore, often superimposed on those of the disease itself. However, it must be remembered that both jaundice and acute abdominal pain may be present without gallstones. During the crises the splenic enlargement increases and the organ may become tender.

On *physical examination* the main feature, aside from evidences of anemia and jaundice, is the enlarged spleen. The degree of enlargement is usually moderate, the edge being one to three inches below the costal margin. In some cases it is huge. It is firm and smooth, and preserves its usual shape. The liver is also moderately enlarged and hard. The lymph nodes are not enlarged.

The *blood* shows a distinct anemia as a rule, but usually of moderate degree only. The red cell count ranges from 3.0 to 4.5 millions, with an average of 3.34 millions (Krumbhaar).[1] The color index is about 1.0. The individual red cells tend to be a little small in diameter, and are dark in color. Poikilocytosis is negligible, and anisocytosis is moderate only. Macrocytes are rare or absent. There are moderate numbers of polychromatophilic red cells, and occasionally a normoblast may be found. Platelets are numerous. During the crises there is a neutrophilic leucocytosis.

[1] Krumbhaar, E. B., Osler's *Modern Medicine*, 1927, Ed. 3, v, 177.

Special methods of examination show striking and characteristic abnormalities in the red cells.

(1) Their shape is abnormal. Although the diameter of the cells is decreased their bulk is normal, as shown by the color index and volume index of about 1.0. Therefore, their thickness must be increased. They are more globular than normal red cells. Naegeli regards this as one specific manifestation of the anomaly that constitutes the disease.

(2) The red cells show a *diminished resistance to the hemolytic action of hypotonic salt solution.* Whereas normal cells show no hemolysis until the concentration of salt is reduced to about 0.44 per cent, the cells in this disease may begin to dissolve in concentrations of 0.5 to 0.6 per cent, or even of 0.7 per cent. Complete hemolysis may occur in concentrations of 0.4 to 0.48 per cent as compared with 0.34 per cent for the normal cells.[2] This phenomenon is often referred to as "increased fragility" of the red cells. It has been extensively studied and is believed to be pathognomonic of the disease. It is nearly constant. The degree of increased susceptibility, however, varies a good deal in different individuals, and may vary in the same patient at different times. Not rarely the difference is relatively slight, less than 0.1 per cent above the normal figure. In most other anemias, and also in obstructive jaundice, the resistance of the red blood cells is somewhat increased.

[2] Kolmer, J. A., "Technique of Fragility Tests," *Infection, Immunity, and Specific Therapy,* 1915.

(3) There is usually a great increase in the number of reticulocytes. These cells are somewhat increased in any anemia with active blood regeneration, but while in most forms of anemia these cells make up from 1 to 5 per cent of the red cells, in hemolytic jaundice they often are increased to 10 to 20 per cent, and sometimes even to 50 per cent, of the red cells. While there is considerable variation in the number of reticulocytes, outspoken cases at some time usually show a very high percentage, more than are commonly seen in any other condition.

In outspoken cases the disease runs a *chronic course* with about three or four crises of hemolysis each year. During these periods the patients are acutely sick and confined to bed. The crises gradually subside after 3 to 4 weeks, the symptoms of illness lessen or disappear, and the jaundice fades. Hemolysis continues at a rate which is much reduced, but is still somewhat above normal. During the remissions patients with a severe form of the disease are still largely incapacitated for work, and the weakness, together with the symptoms of gallstones which are so frequently present, makes chronic invalids of the patients. However, in the majority of cases the symptoms are relatively mild. There is not much tendency for the disease to become more severe, but the attacks tend to get milder with increasing age. Death from the disease itself is distinctly rare in the familial type, although it may occur. Latent cases have been reported, recognizable only by the susceptibility of their cells

to hypotonic salt solution. The frequency and significance of such cases has not yet been determined.

Pathological Anatomy. The pulp of the enlarged spleen is enormously engorged with blood. There is but little fibrosis. There is a moderate increase in pigment, and there are moderate numbers of macrophages containing red blood cells in the spleen and also in the liver and marrow. The bone marrow is markedly hyperplastic.

Pathogenesis. There is still dispute as to whether the primary factor in the accelerated blood destruction is an inherent weakness in the red cells or a pernicious hemolytic activity on the part of the spleen. The brilliant therapeutic results that follow splenectomy have suggested the latter alternative. However, it is generally conceded that the red cells are defective and presumably may be abnormally susceptible to the normal processes of blood destruction. Furthermore, the increased fragility and the microcytosis persist after splenectomy, though in lessened degree, according to most observers.

Differential diagnosis. The diagnosis depends on the demonstration of the four cardinal features of the disease: the familial history, the hemolytic crises with hematogenous jaundice, the diminished resistance of the red blood cells to hypotonic salt solution, and the enlargement of the spleen. In individual cases any one of these features may be lacking. Difficulties may arise in the following conditions:

(1) Other forms of *anemia*, chiefly certain cases of pernicious anemia. The macrocytosis, the absence

of an increased fragility of the red cells, and the history make differentiation simple in most instances, but there may be real difficulties in distinguishing between pernicious anemia and certain cases of the acquired type of hemolytic jaundice.

(2) Other forms of *jaundice*.

Obstructive jaundice due to gallstones may be distinguished by the later onset, absence of anemia, and absence of splenic enlargement. However, gallstones frequently occur in patients with hemolytic jaundice, and symptoms from them may so dominate the clinical picture that the underlying disease is overlooked.

Infectious jaundice may resemble one of the crises of hemolytic jaundice, but is distinguished by the history and the absence of the characteristic blood changes.

(3) Other diseases associated with *splenomegaly*.

Banti's disease (splenic anemia). In this disease jaundice is usually insignificant or absent, the color index is low, there are few reticulocytes, and no increased fragility of the red cells.

Cirrhosis of the liver, with jaundice, and *syphilis* may be distinguished by the history and the absence of the blood changes.

Treatment. In the milder familial cases no special treatment is necessary. In the cases with severe symptoms splenectomy is the only effective measure, and in substantially every case it is followed by a symptomatic cure. There is immediate cessation of hemolysis, with disappearance of jaundice; relief from symptoms, and improvement in the anemia. The relief is usually permanent. One

patient was well 27 years after operation (Piney). The fragility of the red blood cells usually persists in some degree, however. The postoperative mortality reported by Giffin [3] was 5 per cent. According to him, the operation should be done during a remission, and not during a crisis. Transfusions should usually precede and follow the operation. Gallstones if present should be removed.

Radiation of the spleen has not been effective. Iron and arsenic are of no value. Successful treatment of associated diseases has little or no effect on the course of the familial type of the disease, although without doubt fatigue, exposure, or intercurrent disease may precipitate or increase the severity of individual crises.

Acquired Hemolytic Jaundice is distinctly less common, and the cases do not form a clear-cut clinical entity as do those of the familial type. Both types have in common the crises of hemolytic jaundice, the characteristic blood changes, and the enlarged spleen. In the acquired type symptoms usually appear at a later age than in the familial form. Cases developing after the third decade are all acquired. Acquired cases fall into two groups: (1) "idiopathic" cases, in which no adequate exciting cause can be discovered; and (2) cases in which the disease syndrome is secondary to some infection or intoxication. Among the conditions which have been reported as causes of the acquired type of the disease may be mentioned syphilis, malaria, tubercu-

[3] Giffin, H. Z., "Splenectomy," *Surg. Gyn. and Obst.*, 1927, xlv, 577.

losis, dysentery, hookworm infestation, cholangitis, sepsis, toxemia of pregnancy, cirrhosis of the liver, carcinoma of the gall bladder, intestinal obstruction, and leucemia. The diversity of this list and the great rarity with which the syndrome of hemolytic jaundice is associated with any one of these causative diseases indicates clearly that there must be some individual peculiarity which predisposes to the development of the syndrome. It has been suggested that these patients are really latent cases of the familial type, and that the associated disease merely precipitates the development of the symptoms. The weight of evidence, however, is against this view. The two types are probably quite distinct.[4]

The acquired form differs from the familial type in the following points: It is usually more severe in every way. The crises are more acute, and often more protracted, and the tendency to remission is less. The anemia is more marked, and may be profound. The average red cell count is about 2.0 millions (Krumbhaar), and may fall to 500,000. The jaundice tends to be less marked, and the diminished resistance of the red cells is often less pronounced (in vitro). Microcytosis is also less definite. Patients with the acquired type do not transmit the disease to their children.

Treatment consists primarily in recognizing and treating the underlying disease, and the prognosis depends largely on the seriousness of that disease, since cure of the latter results in recovery from

[4] Tileston, W., "Hemolytic Jaundice," *Medicine*, 1922, i, 355.

the hemolytic jaundice also. If no cause can be found the outlook is unfavorable. Splenectomy has been tried, and has been successful in some cases but not in all. Treatment is otherwise purely supportive and symptomatic.

CHAPTER XVI

SPLENIC ANEMIA (BANTI'S DISEASE)

SPLENIC anemia is a term sometimes applied to an ill-defined group of cases characterized by anemia, enlargement of the spleen, and a normal or reduced leucocyte count. It is not a distinct disease, but merely a symptom complex. Many of the cases thus designated are really patients in the initial stage of Banti's disease, and if the use of the term splenic anemia is justified at all, it should be restricted to this conditon.

The *etiology* of Banti's disease is unknown. It is often attributed to a chronic infection. Young adults are chiefly affected.

Symptoms. During the first stage of the disease, which may last for several years, there is increasing weakness, pallor, digestive disturbances, and enlargement of the spleen. There is a marked tendency to profuse gastric hemorrhage, which is a frequent cause of death. The disease runs a slowly progressive, chronic course. After a variable period there may develop some enlargement of the liver, which is followed after a few months by atrophic cirrhosis of the liver, ascites, jaundice, increasing anemia, and emaciation. Death usually follows within about a year.

The *blood* shows the changes of a secondary anemia. The anemia may be fairly marked, but

is rarely extreme, 50 per cent of hemoglobin and 3 million red cells are average figures. There are but few signs of blood regeneration; no normoblasts, few if any polychromatophilic red cells, and few reticulocytes. The platelets tend to be a little reduced. The white cells are normal, or there is an absolute and relative reduction in the neutrophile leucocytes. These features indicate an aplastic, or at least an inactive, marrow. Some cases show signs of increased blood destruction.

Differential diagnosis. The disease may be differentiated from hemolytic jaundice by the absence of the hemolytic crises, and by the normal resistance of the red cells to hypotonic salt solution. These cases must be distinguished also from ordinary atrophic cirrhosis, on the one hand, and from chronic infections of recognized type (such as syphilis and malaria) with enlarged spleen, on the other. In some cases this is impossible, and this difficulty has led certain authorities to deny the existence of Banti's disease as a distinct clinical entity.

Treatment. The prognosis is unfavorable. The most effective therapeutic measure is splenectomy. If carried out early there is often symptomatic cure that may be maintained for several years at least. The operative risk is greater and the end results are less favorable than in hemolytic jaundice. In the cirrhotic stage little can be accomplished. Otherwise treatment is purely symptomatic.

CHAPTER XVII

ANEMIA PSEUDOLEUCEMICA INFANTUM (VON JAKSCH'S ANEMIA)

THIS condition is not a distinct disease, but a symptom complex which may appear in infants and young children in a variety of pathological conditions. It is characterized by the gradual development of a severe anemia of the secondary type with many nucleated red blood cells, by a leucocytosis with myelocytes, and by enlargement of the spleen.

Etiology. The condition rarely appears after the third year, and is most frequent between the third and sixth months. No single etiological cause has been demonstrated. A large number of the cases show evidence of improper diet, malnutrition, gastrointestinal disturbances, or rickets, and these conditions are probably important underlying causes. Some cases have been attributed to syphilis, tuberculosis, or other chronic infections. It is regarded by Naegeli and many others as a peculiar infantile type of response to an intense marrow stimulation, such as might occur in a severe secondary anemia from any cause.

Symptoms. The onset is insidious, and the course as a rule is chronic, lasting for months and even years. The symptoms are those of anemia, together with those of the underlying disease—fatigability,

weakness, and prostration in varying degree, together with malnutrition and gastrointestinal disturbances. The blood changes are the most constant feature of the condition. The red cells are much reduced—in the most marked cases they may be as low as 500,000 per c.mm. The color index is usually below 1.0. There are many polychromatophilic and stippled cells, and many mature and immature normoblasts (macroblasts). Typical megaloblasts rarely, if ever, occur. The leucocyte count is usually between 20,000 and 40,000, but may rise to over 100,000. Myelocytes are constant, but not often numerous. Neutrophiles usually predominate, but in some cases lymphocytes are more numerous.

At autopsy the striking feature is the hyperplasia of the erythropoietic tissue, which is abundant in the spleen, liver, and lymph glands, as well as in the marrow.

Diagnosis. The main problem is the recognition of the underlying disease. These cases have been mistaken for myeloid leucemia, and also for pernicious anemia. The resemblance is but slight in either case. Pernicious anemia is practically unknown at this age, and the blood changes are quite different. Myeloid leucemia in young children is an acute disease, and the blood shows large numbers of immature cells, usually many myeloblasts, as well as myelocytes. The possibility of familial hemolytic jaundice must be kept in mind.

Prognosis and treatment. The average mortality is stated to be about 20 per cent. A majority of the cases recover completely. The outlook in an in-

dividual case depends on the possibility of recognizing and treating the underlying disease. General hygienic and dietetic measures are of chief importance, with special attention to the possibility of rickets. Iron and, more particularly, arsenic have apparently been of benefit in some cases. Transfusions should be given in severe cases. Favorable results have been reported following splenectomy in some cases. However, such a procedure is not to be recommended indiscriminately in this heterogeneous group of conditions.

CHAPTER XVIII

POLYCYTHEMIA

BY POLYCYTHEMIA is usually understood an increase above the normal in the number of red blood cells per c.mm. of blood. *Erythrocytosis* is a more precise term for this condition. A polycythemia may be relative or absolute.

A Relative Polycythemia, or simple erythrocytosis, is a condition in which the number of red blood cells per c.mm. is increased, but the total number of these cells in the body is normal. Such a condition is usually a transient phenomenon resulting from a diminution in the volume of the plasma which occurs with dehydration. It is seen especially in case of severe vomiting, diarrhea, sweating, exudation of fluid from the lungs following certain forms of gas poisoning, and water starvation. It disappears promptly with an adequate fluid intake.

Abnormalities in the red cell count in the peripheral blood may be due to irregularities or variations in the distribution of cells in the different vessels. Thus, a misleading erythrocytosis may be found in otherwise normal individuals if blood for the count is obtained from an area of localized cyanosis, such as is often seen in the ear lobe or finger tip, particularly if cold. In such cases blood from a vein will show a perfectly normal count. Another cause of sudden variation in the red cell

count of normal individuals is to be found in the storage of red blood cells in the spleen, or in the sudden outpouring from this organ, and perhaps from other reservoirs, of large numbers of erythrocytes in response to some sudden stimulus.

Plethora is a condition in which the blood volume is increased, but the concentration of red cells in the blood is normal.

An absolute polycythemia is a condition in which there is an increase in the total number of red blood cells in the body, as well as in the concentration of these cells in the blood. This is of far greater significance than a relative polycythemia, since it indicates the presence of some abnormal condition which stimulates the marrow to an overproduction of cells. Actual measurements of blood volume are, therefore, desirable in all cases of polycythemia.

An absolute polycythemia occurs (1) as the most characteristic symptom of the specific disease known as polycythemia vera (or rubra), or preferably as erythremia, and (2), more frequently, as a symptom of a variety of other conditions. Among these other conditions may be mentioned:

(1) *Normal individuals living at high altitudes.* An appreciable increase in the red blood cell count may occur within a few hours if the change in altitude is made rapidly, as in aviation. This is not accompanied by signs of new blood formation, and must be due to a redistribution of cells in the circulation, to an outpouring from the spleen and other internal organs. The increase in such cases rarely exceeds a half million cells per c.mm. A

more significant rise occurs only after some days' sojourn at the high altitude, and is accompanied by an increase in reticulated red cells and other evidence of accelerated blood formation. In marked cases the count may eventually reach 7 or 8 millions. There is great individual variation in the degree of the increase, and in many cases it is insignificant or slight. It has been shown pretty conclusively that the low oxygen tension prevailing at high altitudes is the stimulus which arouses the bone marrow to increased activity. Return to sea level is followed by a return of the blood to normal. Prolonged life at high altitudes may result in the development in some individuals of the typical clinical and pathological picture of polycythemia vera.

(2) *Myocardial insufficiency*, with chronic cyanosis. In adults this is most often seen in mitral stenosis, particularly in cases in which long continued chronic passive congestion of the lungs has resulted in considerable fibrosis. The rise is usually moderate only. The most marked erythrocytoses in this group occur in cases of congenital heart disease, especially in pulmonary stenosis. Counts of 7 to 8 millions are not uncommon, rarely even higher.

(3) A large group of *chronic pulmonary diseases*, such as emphysema, chronic pulmonary fibrosis, either tuberculous or nontuberculous in origin, stenosis of the air passages, and any condition which interferes with pulmonary ventilation. A special group of such cases has been described as Ayerza's disease, associated with sclerosis of the pulmonary artery. In the late stages of all these

conditions myocardial insufficiency with right ventricular failure is prone to develop. As a result a marked degree of chronic anoxemia develops. The erythrocytosis is often marked, even 8 to 10 millions per c.mm. The spleen may be enlarged, and the nervous and other symptoms seen in polycythemia vera may be present.

(4) *Chronic poisoning* with a variety of drugs, chiefly blood poisons which, in appropriately small dosage, may act as irritants to the erythropoietic tissue. Among these may be mentioned arsenic, phosphorous, mercury, radium, gum shellac, and some benzol derivatives.

(5) After *recovery from any severe anemia* a transient erythrocytosis may occur—a manifestation of overcompensation.

ERYTHREMIA, OR POLYCYTHEMIA VERA

Erythremia is a chronic disease of unknown etiology characterized by a great increase in the number of circulating red cells and hemoglobin, by a peculiar intense flushing of the skin and mucous membranes, by weakness and nervous disturbances, and by enlargement of the spleen. While the disease was described by Vaquez in 1892, it was first brought to general notice by the descriptions of Osler. It is a disease of late middle life, and occurs about equally in men and women. A number of familial cases have been reported.

The onset of *symptoms* is insidious, and the course is usually slowly progressive, although there may be partial remissions. The initial symptoms are usually weakness and fatigability. These are soon

followed by a variety of nervous symptoms. Headaches are common, sometimes severe, and may be associated with increased pressure in the cerebrospinal fluid. There may be fullness in the head, dizziness and vertigo, or actual syncopal attacks. Mild mental disturbances are common: irritability, depression, emotional instability, loss of memory, and particularly inability to concentrate and increasing inaptitude for physical and mental work. Sensory disturbances are common, especially roaring in the ears, transient visual disturbances, and numbness and tingling or burning sensations in the fingers or feet, resembling those seen in pernicious anemia. There is often great sensitiveness to cold. Neuralgic pains are common, as are pains in the bones, which have been attributed to the hyperplastic bone marrow. Nausea and vomiting occasionally occur. Constipation, emphasized by Osler, is not constant. Minor digestive disturbances, such as fullness, epigastric pain, and belching, are common. Large gastric hemorrhages occasionally occur. More or less dyspnea on exertion is usually present. There may be palpitation, cardiac oppression, or all the symptoms of outspoken myocardial insufficiency.

On *examination* the striking feature in most cases is the congestion of the skin and mucous membranes. This is quite different from the bluish tint seen in the ordinary cyanosis of myocardial insufficiency. The color of the skin is an intense mottled brick red, marked on the face, especially the cheeks, nose, and ears, and on the extremities, but not as rule on the trunk. The visible mucous membranes have an intense purplish-red, plum color, more livid than

that of ordinary cyanosis. The color is due to an unusual dilatation of the capillaries. Not only are the individual vessels distended, but there are far more capillaries patent and containing blood than in normal individuals. The discoloration of the skin may be the first thing noticed, and may antedate by years any actual discomforts. In some cases the color is not markedly abnormal. The retinal vessels are markedly congested.

There is often, but not always, a *hypertension.* With this there may be a moderate degree of cardiac dilatation and hypertrophy. The urine as a rule contains a trace of albumin and a few casts. There may be moderate impairment of concentrating power. There is often a marked peripheral arteriosclerosis.

The *spleen* is enlarged in at least three-fourths of the cases. The enlargement is usually moderate, the edge three to four fingers' breadths below the costal margin. It is sometimes more marked than this, but is rarely huge. The surface is hard and smooth. Small infarctions in the spleen are fairly common, with pain, tenderness, and sometimes an audible friction rub. The liver is moderately enlarged in about half the cases. A moderate degree of cirrhosis is not infrequently found at autopsy. Less often there may be clinical signs of cirrhosis manifest.

The *basal metabolic rate* is definitely increased in many cases, but not in all, recalling the increase seen in leucemia. With reduction of the plethora by artificial measures there is in some cases a return of the rate to normal.

The *blood* shows an increase in the number of red corpuscles which at some stage in the disease usually exceeds 8 millions. The count may reach much higher figures, even 12 to 15 millions, but such figures are rarely met with and represent about the maximum number of cells which it is physically possible to compress into 1 c.mm. volume. However, counts as high as 8 millions are by no means constantly present, and in undoubted cases of the disease the count may be between 5 and 6 millions. The hemoglobin is also increased, usually to 19 to 24 grams per 100 cc. (120 to 150 per cent) or higher. The color index is below 1.0, and the cells tend to be a little small and pale. There is apt to be slightly more than the usual variation in size of the red cells. There are generally a few diffusely polychromatophilic cells and a slight increase in reticulocytes. There may be an occasional normoblast, but they are not common.

There is usually a moderate neutrophilic leucocytosis of 10,000 to 15,000, occasionally much higher. There are often a few myelocytes present, and in exceptional cases they may be so numerous as to suggest leucemia. The blood platelets are usually increased, often decidedly so.

The *blood volume* is markedly increased, in extreme cases to more than double the normal value. This is due to the increased volume of red cells, the plasma volume remaining about normal. As a result of the combination of an increased blood volume and an increased concentration of hemoglobin per unit volume, the total quantity of circulating hemo-

globin is often doubled and may be more than three times the normal amount.

The *viscosity* of the blood is greatly increased proportionate with the degree of erythrocytosis. It has been reported as five times or over that of normal blood. The association of such a great viscosity with a large blood volume tends to increase greatly the work of maintaining the circulation, and results in a marked stasis in the capillary bed, especially in the extremities. This slowing of the circulation is an important factor in the production of many of the symptoms of the disease.

Course. The disease is incurable. It is extremely chronic, and runs a slowly progressive course to end eventually in death. However, death is usually due to some associated or complicating condition, rather than to the disease directly. The average duration of life after the development of symptoms is about 6 to 8 years. However, there is good evidence that, in many cases at least, the erythrocytosis exists for years before symptoms appear. Not infrequently the change in color is the only complaint, the patient is "more red than sick." After symptoms have once developed the patients are more or less incapacitated, and often feel quite weak and miserable, except as their condition may be improved by treatment. The prognosis must be guarded in any single case, as death may occur early from one of the *complications.* Among the latter may be mentioned the following:

Hemorrhages are common. They may occur as an insignificant oozing from the mucous membranes, or they may be exsanguinating. They are one of

the common causes of death. The commoner sites of bleeding are the nose, gums, stomach, intestines, lungs, and uterus. Cerebral hemorrhages are common. The bleeding is not associated with any defect in blood coagulation, but is attributable to the great distension of the vascular system. A striking feature in those surviving a severe hemorrhage is the extraordinary rapidity with which blood is regenerated.

Thromboses are common and may occur in almost any veins. They may occur in the cerebral vessels, in the portal vein or its branches, or more rarely in the mesenteric vessels.

Increased susceptibility to infections exits and accounts for many deaths. Of those patients who survive for some years after the onset of symptoms many develop hypertension, cardiac dilatation, and die from *myocardial insufficiency*. A smaller number show signs of chronic nephritis and die of uremia. A certain number die from cirrhosis of the liver.

In rare cases which have escaped these complications there may come a stage when the marrow becomes exhausted. The erythrocytosis then gives place to an anemia of an aplastic type which may resemble pernicious anemia. In some of these cases there may be such an outpouring of granular leucocytes and myelocytes as to simulate myeloid leucemia.

Pathogenesis. The essential pathological anatomical change found in erythremia is the extreme hyperplasia of the erythropoietic tissue of the marrow, with overcrowding of the peripheral circula-

tion with red cells. Two main hypotheses have been advanced to explain this hyperplasia. According to one of them, erythremia is to be regarded as quite analogous to leucemia, as a primary disease of the marrow, related perhaps to the malignant tumors. The other theory regards the hyperplasia as a physiological response to some intense stimulus. It was pointed out by Osler that the symptoms of this disease bear a striking resemblance to those of mountain sickness. The pathological anatomy and physiology is practically identical with that seen in mountain sickness and other conditions associated with marked anoxemia. This has suggested that in erythremia there may be an *anoxemia of the tissues*, such as might arise if there were some disturbance to the free diffusion of oxygen either from the pulmonary alveoli into the red cells, or from the red cells into the tissues. Such an anoxemia, if present, would not only explain the peculiar character of the clinical symptoms, but would be an effective stimulant to the marrow. This theory is attractive, but as yet has no conclusive evidence to support it, although Harrop and Heath have shown that there is some retardation in the diffusion of oxygen from the lungs into the red cells.[1]

Diagnosis. The diagnosis of erythremia is usually suggested by the peculiar color of the patient, and is confirmed by the characteristic clinical symptoms, by the enlargement of the spleen, and by the demonstration of an erythrocytosis, together with the exclusion of other conditions which may cause chronic cyanosis and (symptomatic) polycythemia.

[1] Harrop, G. A., "Polycythemia," *Medicine*, 1928, vii, 291.

The diagnosis should be made with great caution if the red cell count is under 6 millions, and only if the other manifestations of the disease are definite. The demonstration of an increase in blood volume is of great significance in doubtful cases.

Treatment is palliative and symptomatic. The activities of the patient should be curtailed so as not to exceed his endurance. Measures effective in reducing the plethora and erythrocytosis give temporary symptomatic relief, often in marked degree. This is probably due to a lessening of the peripheral congestion and an acceleration of the circulation rate, which results from the reduction of the viscosity of the blood.

Repeated *venesections* usually give much relief at first, but the improvement is only transient, and the procedure doubtless results in further stimulation of the marrow. Venesection may be advisable to get quick relief. The quantity should be from 500 to 600 cc. Smaller amounts are useless, and larger amounts may be dangerous, especially in symptomatic polycythemias.

Radiation of the long bones with X-ray or radium gives more lasting relief and is one of the most valuable methods of treatment. The exposures must be given cautiously, carefully controlled by frequent blood counts. Excessive radiation may cause a fatal aplasia of the marrow. There is serious question as to the advisability of radiating the spleen. Theoretically nothing should be done which would lessen its hemolytic activity or its inhibitory effect on the marrow.

Splenectomy is theoretically contraindicated for the same reasons, and the results thus far reported in a few cases are mostly unfavorable.

Of drugs which reduce the red blood cell count *phenylhydrazin hydrochloride* has given the most satisfactory results.[2] The drug is a powerful and dangerous poison, and should not be used unless its action is thoroughly understood, and unless the patient is under constant observation, and complete blood counts can be made at least every second or third day. The drug acts by destroying the circulating red cells, and probably also by injuring and depressing the hemopoietic tissue of the marrow. The drug is slowly excreted and its action is cumulative, persisting for from one to two weeks or more after administration is stopped.

The drug is administered by mouth, preferably in capsules, in a dose of 0.1 gram. The total dosage for a course varies from 1.5 to 6.0 grams, and is usually between 2 and 4 grams. In a conservative method of administration the maximum daily dose should not exceed 0.3 gram, and after a total of 1.2 grams have been given the dose should be reduced to 0.1 gram per day. With such dosage a week or more may be required before much action of the drug is manifest. More intensive treatment with the drug to get quick relief from symptoms is dangerous. Venesections should be used if necessary for this purpose.

The first evidences of its activity are: (1) an increase in the leucocyte count, (2) an increase in the

[2] Brown, G. E., and Giffin, H. Z., *Arch. Int. Med.*, 1926, xxxviii, 321.

bilirubin in the serum (positive indirect Van den Bergh reaction), (3) the passage of very dark-colored urine, due to the presence of some as yet unidentified pigment, and (4) a beginning fall in the red cell count and hemoglobin. In patients with only moderately high counts the drug should be stopped at this point, since there is usually a continued fall in the red cell count after the drug is discontinued of from 1 million to 2.5 million cells per c.mm. In no case should it be continued after the red blood cell count has fallen to 5 millions. If given cautiously in this way the red cell count can gradually be reduced, usually with little discomfort to the patient.

If the treatment is too energetic there will be a rapid destruction of red cells, with all the evidences of an acute hemolytic anemia; outspoken jaundice, weakness, anorexia, vomiting, hematemesis, hematuria, and the rapid development of a profound anemia. There is a possibility of renal and hepatic injury. In the cases thus far reported there has been a fairly rapid recovery from such an anemia with no permanent ill effects.

In nearly all cases treated there has been striking relief from symptoms, and patients are often able to return to work. Hypertension, if present, is usually reduced. The relief is temporary, and after 3 to 6 months, and sometimes less, the red cell count rises and symptoms return. In such cases second and third courses usually have given relief, but not always as satisfactorily as did the first. It is probably preferable to try to keep the blood at approximately a normal level by the continuous ad-

ministration of small doses of phenylhydrazin: from 0.1 to 0.3 gram per week. The average quantity required in Owen's cases was 0.15 gram per week. A return of symptoms is usually an indication for increasing the dose or resuming the drug, even though the red cell count may not be extremely high.

How long such improvement can be maintained, to what extent tolerance to the drug may be developed and larger doses be required, and to what extent the liver may be damaged by continued treatment, only further observations can tell. In general, the results appear as promising as those of any other form of treatment, except possibly radiation of the long bones. It should again be emphasized that neither of these measures can safely be undertaken unless it can be controlled carefully by frequent blood counts.

CHAPTER XIX

THE LEUCEMIAS

Definition. Leucemia is a disease characterized by an abnormal proliferation of one of the leucopoietic tissues (myeloid or lymphoid), and by the appearance in the circulating blood of immature types of white corpuscles not normally present there. There is usually a marked increase in the number of circulating white corpuscles.

Classification. There are two distinct types of leucemia, myeloid and lymphoid (or lymphatic). In myeloid leucemia (*myelosis*) the hyperplasia involves primarily the leucopoietic tissue of the marrow, and the predominant cells in the blood are the granular leucocytes and their precursors. In lymphatic leucemia (*lymphadenosis*) it is the lymphoid tissue which is hyperplastic, and the predominant cells in the blood are lymphocytes. The weight of evidence is strongly against the occurrence of "mixed leucemia," in which there is simultaneous involvement of the two systems, or of true transitional forms. The difficulty occasionally met with in differentiating clinically between the two types of the dieases is due merely to a superficial similarity of the immature cells of the two tissues (myeloblasts and lymphoblasts). Much confusion will be avoided if it be remembered that the two leucemias are tissue diseases, not organ diseases. The tissue

affected is hyperplastic, wherever it may be found in the body. The occasional occurrence of hyperplastic lymphoid tissue in the bones, or of myeloid tissue in the lymph glands, does not justify one in classing a case as "mixed" or assuming a transformation ("metaplasia") of one tissue into the other.

Each type of leucemia may be subdivided into *acute* and *chronic* forms, according to the character of the onset and the duration of the disease. Such a subdivision is arbitrary, as every gradation between them occurs. In general, cases with an abrupt onset, running their course within three or four months, may be classed as acute, and those of longer duration as chronic.

Etiology. Nothing whatever is known as to the cause of the disease. It is relatively rare. It is more common in men than in women. It occurs in all races, and a similar condition has been found in several other mammals and in birds.

The *pathogenesis* of the disease is also uncertain. The two most plausible theories are (1) that it is due to some infection or (2) that it is a peculiar form of neoplasm. The adherents of the hypothesis that leucemia is an infection find their chief support in the resemblance of the acute leucemias to acute sepsis, and in the fact that the leucemia of fowls can be transmitted by cell-free Berkefeld filtrates of the affected tissues. Such transmission has not been successful in leucemia in mammals. Furthermore, as chicken sarcoma can be transmitted from one bird to another in a similar manner (Rous), such transmissibility of leucemia in birds

would not of itself exclude a relationship to the neoplasms. On the other hand, the majority of cases of leucemia differ from the neoplasms in some fundamental particulars. The hyperplasia involves simultaneously substantially the entire mass of the affected tissue in widely separated areas, and does not spread from a localized primary area of growth. In leucemia there is rarely any distinct tendency to penetrate organ capsules and invade other tissues, and metastases do not occur.

CHRONIC MYELOID LEUCEMIA

Chronic myeloid leucemia is the most common type of the disease. It is a disease of adults, rarely seen under 20. The leucemias that occur in children are acute. The onset is usually insidious. The first symptoms noticed are generally due to the great enlargement of the spleen. The patients may complain of swelling of the abdomen, of a lump in the left side, or of a heavy dragging feeling in the side. Not infrequently there is pain in the region of the spleen from a perisplenitis due to an infarction. While the pain is usually relatively mild, it may in some cases be acute and associated with rigidity and spasm of the abdominal muscles, and give the clinical picture of an acute peritonitis.

Less often the first symptoms may be loss of weight and strength, and increasing pallor. Sooner or later these symptoms always develop, but probably only after the disease has existed for many months. As the disease becomes advanced the emaciation, anemia, and disability become marked. Dyspnea and palpitation are common, often asso-

ciated with cardiac dilatation, edema, and ascites. There may be slight fever, cough, and night sweats, suggesting tuberculosis, which is not rare as a complication. Gastrointestinal disturbances appear, nausea and vomiting, anorexia, diarrhea or constipation.

Hemorrhages frequently occur as a late manifestation, but are rarely profuse. There may be nose bleed, oozing from the gums, bleeding from the gastrointestinal tract, or subcutaneous hematomata, which have been mistaken for abscesses and incised. Petechiæ may occur in the skin, and are common in the retina. They may cause disturbances of vision. Among the less common symptoms may be mentioned disturbances of hearing, itching of the skin, priapism, and pain and tenderness in the long bones.

On *examination* the striking findings are the enlargement of the liver and spleen. The spleen is usually very large, and is often enormous, reaching to the symphysis, or even to the right anterior superior spine, and filling two-thirds of the abdominal cavity. The surface is smooth and hard. Over a recent infarct it may be tender, and a friction rub may be audible or palpable. The spleen retains approximately its normal shape, despite its size. The notches can easily be felt, and can often be seen projecting against the thin tense abdominal wall. The liver edge is usually from 1 to 6 inches below the costal margin, sharp and firm, and its surface is smooth.

The lymph nodes are usually not enlarged except in the end stages, and then only to a slight de-

gree. The skin may show petechiæ, but no nodular infiltrations. The lungs may show signs of a chronic bronchitis. Pleural effusions occasionally occur, especially on the left side. The heart may be dilated, and systolic murmurs are common. The urine usually shows albumin and casts in moderate quantities. Uric acid excretion is greatly increased. The basal metabolic rate is accelerated, roughly in proportion to the activity of the disease process.

Blood. In most cases the striking feature is the *extreme* degree of the *leucocytosis*. The total leucocyte count is usually between 100,000 and 500,000. Counts as high as 1.5 millions have been observed but are exceptional. However, the total count may be only moderately increased or even be within normal limits. The essential feature is not the hyperleucocytosis, but the presence in the blood of considerable numbers of immature myeloid cells, chiefly myelocytes, with usually an occasional premyelocyte and myeloblast (Plate I).

The *polymorphonuclear neutrophiles* as a rule predominate, constituting from 30 to 65 per cent of the leucocytes. The majority are mature cells, essentially normal in appearance, with segmented nuclei and typical neutrophilic granulations. There are always some individual cells which are more or less pathological. There may be giant leucocytes, with many segments in the nucleus, or dwarf forms scarcely larger than a red cell. In some cells the granules may be sparse and stain abnormally. Every transition is present from the typical mature cell to the myelocyte.

The *myelocyte* is the characteristic cell of chronic

myeloid leucemia. These cells are usually a little less numerous than the mature neutrophiles, and make up from 20 to 50 per cent of the total leucocytes. Exceptionally they may be extremely numerous (70 per cent), or they may be well under 5 per cent. Most of the myelocytes have fairly abundant, well-differentiated neutrophilic granulations, but there are usually some with sparse bluish staining (immature) granules, and an occasional myeloblast may be present.

Eosinophiles and *basophiles* are increased in absolute number, and often in percentage as well, and a few eosinophilic and basophilic myelocytes are also present. In an occasional case any one of these types of cells may be enormously increased, even up to 40 per cent of the total leucocytes (*e.g.*, so-called mast cell leucemia). Such cases do not constitute a distinct type of leucemia, but are merely variants of the ordinary myeloid leucemia. *Monocytes* in the early stage of the disease are moderately increased in absolute number, and sometimes in percentage as well. In the later stages, with a very high leucocyte count, an occasional monocyte may still be distinguished, but in general they are lost amid the welter of other cells. In stained specimens their differentiation from immature myelocytes may be difficult. The percentage of *lymphocytes* is greatly reduced, but their absolute number is usually increased.

The *blood platelets* are greatly increased, and careful search may reveal a megakaryocyte, or a fragment of its cytoplasm (Plate II).

The *red cells* and *hemoglobin* fall gradually as

the disease advances, and in the late stages of the disease a pronounced anemia develops. The red cell count as a rule falls to 3 millions, and may reach 1 million. The color index is about 1.0, and the average size and color of the cells is normal. There is a moderate degree of anisocytosis. There are moderate numbers of diffusely polychromatophilic red cells, and the reticulocytes are slightly increased. A highly characteristic and practically constant feature is the presence of moderately *numerous normoblasts*, with a few earlier forms (macroblasts). In no other condition do normoblasts occur in such numbers and with as great regularity. Typical megaloblasts are rare, and are found only in the advanced stages of the disease.

The changes described occur in the great majority of cases, with but slight variations, and the diagnosis cannot be overlooked. Variations from this typical picture will be discussed later.

The *course of the disease* is in general progressive. However, at any period a *remission* may occur, either (1) spontaneously (not frequently); (2) following an acute infection, such as pneumonia (occasionally); or (3) after effective therapy. A remission is followed by an amelioration of the symptoms with increased strength and feeling of well-being, by a rise in red cell count and hemoglobin, a fall in the total leucocyte count, a reduction in the number of immature leucocytes, and diminution in the size of the spleen. In some cases the relief amounts to a symptomatic cure. The spleen may recede from the symphysis to the costal margin, although appreciable enlargement usually

persists. The leucocyte count falls toward normal, and the degree of clinical improvement is roughly parallel to the approach of the count to normal. This may reach normal, or, after radiation, subnormal figures, or it may remain slightly elevated (10,000 to 30,000). In the great majority of cases the differential count reveals the presence of myelocytes in appreciable numbers (5 to 30 per cent). Such a condition is sometimes referred to as *aleucemic leucemia* but the term is misleading since the persistence of myelocytes and usually of normoblasts shows that the blood is still fundamentally "leucemic." The inexperienced observer must not mistake monocytes for myelocytes. In a few cases the myelocytes are reduced to such low figures (1 per cent or less) that the blood becomes virtually normal in appearance. Such a condition may be termed *latent leucemia.*

After a period, which may vary from a few weeks to several months, rarely longer, a *relapse* invariably follows. There may be subsequent remissions, but as a rule these are less complete and briefer than the first, and death eventually occurs. The *average duration* of life after symptoms develop is from 1 to 3 years, and is rarely over 5 years. Cases are on record which have lived 10 years, and even 25 years (cited by Naegeli). At any time in the course of the disease a rapidly progressive acute exacerbation may occur, and lead to death within a few weeks. Unfavorable symptoms which usually herald the approach of such a relapse are:

(1) A rapid aggravation of symptoms, especially weakness, emaciation, diarrhea, and fever.

(2) A fall in red cell count and hemoglobin.

(3) Either a rapid rise in the total leucocyte count, or a rapid fall to subnormal figures, especially in overradiated cases.

(4) The appearance of large numbers of immature myelocytes and myeloblasts.

(5) A reduction in blood platelets with a marked tendency to bleed.

Of these, (2) and (4) are especially significant, and are invariably of unfavorable import, regardless of the course of the total leucocyte count.

Differential diagnosis. If the blood is examined difficulties may arise only in a few rare instances. The disease may be overlooked in the following conditions:

(1) Patients who are first seen during a remission, when the total leucocyte count is normal. The persistence of some myelocytes and normoblasts will usually make the diagnosis evident. In rare cases the diagnosis may be possible only from the history or the subsequent course of the disease.

(2) In rare cases, during the early stage of the disease the total leucocyte count may remain normal, although typical hyperplasia has occurred in the marrow, spleen, etc. (*Aleucemic myelosis.*) Myelocytes are usually present, though their numbers may be small.

(3) Early cases with a moderately high total leucocyte count, but with few myelocytes.

The diagnosis of chronic myeloid leucemia may be made erroneously in:

(1) Hyperleucocytoses due to infection, hemorrhage, etc. There may be many myelocytes, and

even a few myeloblasts present. Such reactions are most often seen in children and young adults, in whom chronic myeloid leucemia is most unusual. The anemia pseudoleucemica infantum falls into this category. The history and examination will usually show some adequate cause for a leucocytosis.

(2) Focal lesions of the bone marrow, especially metastatic carcinoma. Simon reported a remarkable case, often quoted, where accidental crushing of the bones of the leg was followed by the appearance of the blood changes characteristic of chronic myeloid leucemia, but with complete recovery after amputation.

(3) Pernicious anemia. In rare cases in which numerous myelocytes are present the blood may resemble that of leucemia with a low total leucocyte count.

Treatment. The most effective measure for the treatment of chronic myeloid leucemia is radiation, either with X-ray or radium. If properly treated, patients almost invariably respond well to the first series of radiations, unless the disease process is acute or too far advanced. Radiation is useless and may be harmful in acute cases. If the disease is advanced and emaciation and anemia are marked, radiation is less certain to be effective, but even in such cases marked improvement is often obtained. Radiation should be given, as a rule, as soon as the diagnosis is made. The remissions obtained by radiation are generally more complete and of longer duration than others. They usually appear within a few weeks, and last from a few weeks to six months, rarely as long as a year.

Subsequent radiation for the inevitable relapses tends to become increasingly less effective, and eventually ceases to exert any influence on the course of the disease. Treatment of a relapse should begin promptly, before the condition of the patient has deteriorated markedly. Indications for resuming treatment may be an aggravation of the patient's symptoms, progressive enlargement of the spleen, a rising leucocyte count, particularly if it exceeds 100,000, or an increase in the percentage of pathological leucocytes or immature leucocytes, even though the total leucocyte count is not notably increased.

Proper application of radiation is a matter of expert knowledge, and requires careful control by repeated blood examinations. Radiation should be directed primarily to the spleen. The long bones should receive at most but moderate exposures to avoid depressing the activity of the erythropoietic tissue. Too intensive radiation causes violent reactions, and may result in an aplastic anemia, due to exhaustion of the marrow. In extreme cases there may be practically a complete disappearance of the granular leucocytes, a rapidly progressive anemia, and a diminution of the platelets, with a hemorrhagic diathesis. Such a reaction may be acutely fatal. Radiation should usually be stopped when the total leucocyte count approaches normal. The appearance under treatment of those blood changes which indicate an acute exacerbation of the disease is an indication for the cessation of radiation. However, in rare instances a patient with a high percent-

age of myeloblasts may be benefited if there is a high total leucocyte count.

The final exacerbation in patients who have been treated by radiation often begins abruptly and runs a rapid acute course, with symptoms and blood changes that are similar to those of acute myeloid leucemia. When such an acute stage is reached radiation is ineffective and often harmful. Comfort may be increased and life may be prolonged somewhat by repeated blood transfusions. The utmost care is required in their administration, since uncomfortable and disquieting reactions not infrequently occur in leucemic individuals.

It is still uncertain whether radiation actually prolongs life. Naegeli believes that it does, but the statistics of some other observers fail to confirm this. There is no doubt, however, that radiation usually can bring about a satisfactory remission and temporarily restore comfort and ability to work, and that it can do this more regularly, promptly, and effectively than any other measure now available.

Of the drugs which may influence the course of the disease, *benzol* is the most effective. It is inferior to radiation, as it is less certain in its action and more difficult to control. It should be tried if radiation is not available. The drug is given by mouth in capsules containing 0.5 gram each of benzol and olive oil. Two capsules (1.0 gram benzol) are given four times a day until the leucocyte count has fallen substantially. The blood must be examined frequently and the drug stopped as soon as the leucocyte count reaches 20,000 to 30,000, or as soon as there is any notable reduction in the blood

platelets. Several weeks may be required. In favorable cases a temporary remission is obtained. The drug should not be administered while radiation is being given. It is a dangerous poison, and death has been reported in a number of patients from excessive treatment. A favorable response has been reported in a few patients who had become refractory to X-ray therapy.

Arsenic was the drug chiefly used before the effectiveness of radiation was discovered. After protracted administration remissions occasionally occurred, probably a little more frequently than would be expected without the drug. It is inferior to benzol or radiation. It may be used in conjunction with them, or in cases where they are contraindicated. Arsenic should not be given if it causes digestive disturbances.

Aside from these measures, treatment is purely supportive and symptomatic. Splenectomy is contraindicated. There is as yet no evidence that the course of the disease is favorably influenced, and there is no logical reason why this should be expected. The postoperative mortality in the hands of most surgeons has been high.

ACUTE MYELOID LEUCEMIA

Acute myeloid leucemia is much rarer than the chronic form of the disease. It occurs chiefly in children and young adults. The cause is unknown.

The onset is usually abrupt, and sometimes fulminant, with fever, prostration, weakness, and often sore throat. Highly characteristic and almost constant symptoms are an ulcerative or gangrenous

stomatitis and a hemorrhagic diathesis, with purpuric skin eruptions and oozing from the gums and other mucous membranes. There is progressive emaciation and a rapidly developing anemia. The spleen becomes enlarged. The lymph nodes are not enlarged.

The total leucocyte count at the onset may be normal, or moderately increased. There is usually a progressive rise in the total count, which often exceeds 100,000 within a few weeks. The characteristic feature of the blood is the predominance of extremely immature leucocytes, chiefly *myeloblasts*. With these there are nearly always a few myelocytes and transitional forms. In fulminant cases the total leucocyte count may be diminished. These cases of "acute aleucemic leucemia," or better, *leucopenic leucemia*, can be recognized only by the demonstration of myeloblasts in the films. The blood platelets are much decreased. The hemoglobin and red cell count fall rapidly and may reach extremely low figures. The color index is about 1.0. Normoblasts are present, and occasionally megaloblasts. The course is steadily progressive. Death usually occurs within 2 to 4 months, and in the fulminant cases may occur within two weeks.

Differential diagnosis. The onset and course of the disease suggest an acute septic infection, and many cases are so diagnosed, particularly if the total leucocyte count is within the range of an ordinary leucocytosis. In some fulminant cases with leucopenia, the blood changes also may resemble those seen in some rare cases of undoubted sepsis, and the exact relation of these cases to sepsis, on the one

hand, and to chronic myeloid leucemia, on the other, is still an open question. Other conditions for which acute leucemia may be mistaken are (1) simple tonsillitis, (2) noma, (3) scurvy, and (4) purpura hemorrhagica. The association of angina and stomatitis with purpura and bleeding should always arouse suspicion of acute leucemia.

In the earlier literature these cases were all described as acute lymphatic leucemia, because the myeloblasts were mistaken for lymphocytes. Despite Naegeli's clear descriptions, many published cases of acute leucemia are still inaccurately identified as to type, and collected statistics are of no value in determining the relative frequency of the two forms. The writer believes that acute myeloid leucemia is much commoner than acute lymphatic leucemia, despite the usual statements to the contrary.

Some cases diagnosed as acute leucemia are really cases seen in the terminal acute exacerbation of a previously unrecognized chronic leucemia, in whom the chronic disease had been overlooked.

Treatment is without effect. Radiation is contraindicated. Transfusions may temporarily stop bleeding, but they are apt to be followed by disagreeable reactions.

CHRONIC LYMPHOID LEUCEMIA

This type of leucemia is less common than chronic myeloid leucemia. Like the latter it is a disease of adults. The cases in children are acute. The essential lesion is an extreme hyperplasia of

the lymphoid tissue, wherever located, throughout the body.

Symptoms. The onset is insidious. The first manifestation of the disease is often the glandular enlargement. Later there is gradually increasing weakness, loss of weight, and anemia, and the symptoms associated with anemia. The *glandular enlargement* is usually generalized, progressive, and eventually becomes quite marked in degree. It involves such glands as the pectorals and postauriculars, which are rarely noticeable in other conditions. The glands are painless, firm, but not stony hard, discrete and freely movable. They may eventually form large conspicuous swellings, but rarely the huge masses sometimes seen in cases of Hodgkin's disease and lymphosarcoma. The glands never soften or show signs of acute inflammation. The deep glands are also involved. The mediastinal glands may reach such a size as to cause signs of mediastinal tumor. There may be variations from time to time in the degree of swelling, and some groups of glands may be more involved than others, but there is never a primary involvement of one group of glands, with secondary extension to other groups as in Hodgkin's disease and lymphosarcoma. The process is generalized from the start.

The tonsils and lymphoid tissue of the nasopharynx are usually hypertrophied, sometimes to the point of causing obstruction. The spleen is enlarged, but not huge, as in myeloid leucemia. The liver is moderately enlarged, and occasionally huge. Infiltrations not infrequently occur in the skin. There may be pruritus, alone, or associated with

urticarial, eczematous, or pityriasis-like scaling eruptions. Palpable nodules may appear, or even tumor-like masses, especially about the face, which may ulcerate. Swellings may also develop in the salivary and lacrymal glands (Mikulicz' syndrome) or in the breasts. Extensive infiltrations of the mucous membranes of the mouth and throat are not uncommon.

The *Blood* generally shows a marked increase in the total leucocyte count, but this is usually distinctly lower than in chronic myeloid leucemia. The average count is between 100,000 and 200,000 cells, but many cases range between 30,000 and 100,000. Exceptionally it may be very high, from 500,000 to 1.5 millions. The great majority of the cells are lymphocytes, often from 90 to 99 per cent. Even in cases with a total count which is normal, or only slightly increased, the percentage of lymphocytes is generally increased. The great majority of the cells (in the chronic cases) are small lymphocytes, essentially identical with those in normal blood. There is an "endless monotony" of similar cells, as contrasted with the great diversity of types seen in chronic myeloid leucemia. However, a careful study of the lymphocytes shows the presence of minor abnormalities which are of some aid in diagnosis in doubtful cases:

(1) Many of the lymphocytes possess scanty cytoplasm or appear as naked nuclei.

(2) Cells containing azure granules are sparse or absent.

(3) There is always a certain number of immature lymphocytes present, larger cells with more

abundant cytoplasm, and nuclei which take a paler stain and show a finer chromatin structure.

(4) Some of the large lymphocytes (lymphoblasts) contain nuclei which show abnormal indentation or lobulation (so-called Rieder cells).

None of these features is pathognomonic of leucemia. In fact, the occurrence of a large percentage of pathological lymphocytes is not characteristic of chronic lymphatic leucemia, and suggests either an acute form of leucemia or more frequently an infectious lymphocytosis.

The red cells and hemoglobin are at first but little changed. As the disease progresses they are gradually reduced, and in the later stages an outspoken anemia of the secondary type develops. Normoblasts are rare. In some advanced cases lymphoid tissue may largely crowd out the erythropoietic tissue of the marrow and cause a severe aplastic type of anemia. In other cases, also advanced, severe anemia may result from hemorrhage due to a reduction in blood platelets. However, in the early stages, the blood platelets are normal or only slightly reduced. Bleeding early in the disease marks a case as acute.

The granular leucocytes are relatively much diminished, and may be absolutely diminished. Eosinophiles and basophiles are virtually absent. An occasional myelocyte may be present, but such cells are sparse, usually of a relatively "mature" type, and should rarely lead to any difficulty in diagnosis.

The *course* of the disease is slowly progressive. The average duration of life is distinctly longer

than in myeloid leucemia, if the acute cases in children be excluded. There may be periods of several years with but little evidence of progression, and a duration of 5 years and more is not rare. A long period in which leucemic changes exist in the blood and tissues probably often precedes the onset of symptoms and the recognition of the disease. Cabot records one case accidentally discovered in which the disease had been present, untreated, for 16 years, without the appearance of any symptoms. Remissions may occur spontaneously, or as a result of treatment. Recovery is unknown. Death occurs as a result of cachexia, intercurrent infection, or occasionally of bleeding.

Differential diagnosis. The positive recognition of lymphatic leucemia by examination of the blood alone, in cases in which the total leucocyte count is not markedly increased, is more difficult and uncertain than in cases of chronic myeloid leucemia. This is because the histological differences between the lymphocytes of normal blood and the immature lymphoblasts are less striking and distinctive than are those between mature leucocytes and myelocytes, and because lymphoblasts appear in appreciable numbers in nonleucemic conditions more frequently than do myelocytes.

Difficulties in diagnosis arise particularly in the following conditions:

(1) Infections associated with a high lymphocytosis.

(a) Whooping cough and the secondary bronchopneumonias that follow it may show a total leu-

cocyte count of from 30,000 to 100,000 or more, with a high percentage of lymphocytes, chiefly of the small mature type. The history and usual absence of notable glandular enlargement will make the differentiation from leucemia possible.

(b) Infectious mononucleosis is more apt to be confused with the acute forms of leucemia. The cells are chiefly pathological types of large lymphocytes.

(c) Cabot has called attention to a similar high lymphocytosis in some cases of streptococcal wound infections with sepsis.

(d) The postinfectious lymphocytosis that may follow almost any type of infection may occasionally be quite marked and a source of confusion.

(2) Diseases associated with a general lymph glandular enlargement and enlargement of the spleen. In most of these cases the blood examination is decisive as to the existence of leucemia.

(a) Tuberculosis of the lymph glands. Rarely is the glandular enlargement in tuberculosis really generalized, and the spleen is rarely enlarged. Tuberculous glands tend to soften, to become adherent to each other and to the surrounding tissues, especially the skin, which becomes involved in the inflammatory reaction. The lymphocytosis in tuberculosis is usually slight and never confusing.

(b) Hodgkin's disease (lymphogranuloma or malignant granuloma of the German writers) begins in a single group of glands (or aggregation of lymphoid tissue), usually in the upper posterior cervical or supraclavicular group on one side, ex-

tends secondarily to adjacent groups of glands, and then by progressive stages to more distant groups. Only in advanced stages is there a truly generalized glandular involvement. There is more tendency in Hodgkin's disease for the glands to form large masses which cause distressing symptoms from pressure on neighboring structures, especially in the mediastinum. Recurring febrile periods are common in Hodgkin's disease, but rare in leucemia. The blood in Hodgkin's disease is never leukemic, and does not show a lymphocytosis. Histological examination of an excised gland makes the differentiation certain, and should be carried out whenever there is any doubt as to the diagnosis. Microscopical sections show a peculiar type of inflammatory process in which a granulation tissue rich in large epithelioid cells, and giant cells (both derived from monocytes according to Sabin), and eosinophiles, crowds out the lymphocytes of the glands. As a result there may be a reduction in the percentage of lymphocytes in the blood in the late stages. Early in the disease there is often an increase in blood platelets, and a high percentage of monocytes (Bunting). Exceptionally a high eosinophilia has been observed. Late in the disease a severe anemia of the secondary type develops.

(c) Lymphosarcoma (Kundrat). This is a peculiar type of malignant neoplasm in which the cells constituting the growth are lymphocytes. As in most neoplasms the process starts in a localized area, and spreads by direct extension of the growth to neighboring structures, and by metastasis to other lymph glands. Like Hodgkin's disease it forms

large masses which may cause serious symptoms from pressure on neighboring structures. The growth penetrates the gland capsules and invades the surrounding tissues. The individual glands do not remain discrete, but become engulfed in a large mass of tumor tissue. The growth tends to penetrate connective tissue spaces and surround and ensheath organs and vessels, but does not as a rule invade and destroy organs, muscles, or vessel walls, as do most malignant neoplasms. By successive metastases many groups of lymph glands may eventually become involved. In the late stages metastases may occur to the skin, the bone marrow, etc., apparently by way of the blood stream, but the liver and spleen are rarely much involved.

Several types have been distinguished according to the region involved. The group in which the cervical and mediastinal glands are involved may clinically resemble lymphoid leucemia. The blood is not leucemic, and the lymphocytes are not increased. In the late stages they may be diminished. The abnormal cells rarely get into the circulation. However, cases regarded histologically as lymphosarcoma are recorded, in whom later leucemic blood changes developed. Such cases have been described as "*leucosarcoma*," and the existence of such cases has been advanced to support the claim that leucemia is a kind of neoplasm. Microscopic examination of a gland section easily distinguishes lymphosarcoma from Hodgkin's disease, and usually from lymphatic leucemia also, but in the early stages this may be difficult. Lymphosarcoma responds tem-

porarily to radiation more satisfactorily than does lymphatic leucemia.

(d) Aleucemic lymphadenosis (aleucemic lymphoid leucemia, latent lymphatic leucemia) is identical clinically and anatomically with chronic lymphatic leucemia, except that the total leucocyte count is not increased. In some cases the differential count is normal, but often there is a relative lymphocytosis which indicates that the condition is essentially leucemic. The course of the disease is the same as in chronic lymphatic leucemia, except that it may be more protracted, and the symptoms at first may be less pronounced. At any time in the course of the disease the blood may become frankly leucemic. However, some cases have died of the disease before these changes appeared in the blood.

Treatment of chronic lymphoid leucemia. *Radiation* offers the best prospect of obtaining temporary relief. The results are less certain and relief is often distinctly less complete than in chronic myeloid leucemia. In about half the cases a fairly satisfactory remission is obtained. *Benzol* may also bring about a remission. Arsenic may be tried. Treatment otherwise is purely symptomatic. Neither the spleen nor the tonsils and adenoids should be removed.

ACUTE LYMPHOID LEUCEMIA

This type of leucemia occurs in children, but it is excessively rare in adults if terminal exacerbations of a chronic leucemia are excluded. The onset is rapid, often abrupt, with fever, prostration,

headache, anorexia, stomatitis, sore throat, and a hemorrhagic diathesis. There may be gangrenous ulcerations of the tonsils. Clinically it is practically indistinguishable from acute myeloid leucemia, and resembles a virulent acute infection, or in some cases a purpura hemorrhagica. At the onset glandular enlargement is absent or insignificant, but in most cases there soon appears a progressive general glandular enlargement in which the cervical glands take a conspicuous part. The spleen also becomes enlarged.

The *Blood* at first shows a normal or slightly elevated total leucocyte count. This usually rises quite rapidly and may exceed 100,000 within two weeks. In extremely acute cases the total leucocyte count may remain normal or subnormal throughout the course. There is usually a relative lymphocytosis, with a preponderance of immature large lymphoblasts which tend to become smudged in the films. The blood platelets are much diminished. There is a rapidly progressive anemia, with little evidence of blood regeneration. Normoblasts are sparse or absent.

The *course* of the disease is rapidly progressive with little or no tendency to arrest or remissions. Death inevitably occurs, either directly from the effects of the disease, from hemorrhage, or from intercurrent infection. Death may occur within a few days, and usually within 10 or 12 weeks. There is no effective *treatment*. Transfusions may be of temporary benefit.

The *differential diagnosis* may present difficul-

ties, and is of great practical importance because of the hopeless prognosis. The following conditions must be especially considered:

(1) Whooping cough. The lymphocytes are small mature cells, there is no diminution in platelets, and as a rule, no anemia.

(2) "Infectious mononucleosis." The symptoms are much milder, there is no ulcerative or gangrenous stomatitis, there is no anemia, no reduction in platelets, and no bleeding.

(3) Infectious lymphocytoses of other origin, rarely.

(4) Fulminant cases of sepsis with diminution of the granular leucocytes and a relative lymphocytosis. The lymphocytes are usually small mature forms. Blood cultures may be necessary to determine the diagnosis.

(5) Agranulocytic angina. The lymphocytes are chiefly mature small forms. The absence of anemia, persistence of normal platelets, and lack of bleeding should differentiate at least the typical cases in this group.

(6) Purpura hemorrhagica. There is usually no lymphocytosis, and few if any immature lymphocytes. The spleen and lymph glands are not enlarged. In some cases differentiation is impossible (see Aleukia).

(7) Acute myeloid leucemia. The distinction is of no practical importance, but is of scientific interest, and depends on the identification of the immature cells (as lymphoblasts or myeloblasts). In the writer's opinion acute myeloid leucemia is commoner than acute lymphoid leucemia.

SPECIAL TYPES OF LEUCEMIA OR ALLIED CONDITIONS

Chloroma. This rare disease is a peculiar type of leucemia characterized by localized invasive tumor-like growths of leucopoietic tissue. Both myeloid and lymphoid forms have been described, but the majority are clearly myeloid in origin, and possibly all should really be classed as myeloid. In gross the tumor masses as a rule show a greenish color on section. The swellings are usually connected with the bones. The skull is most often involved, particularly the orbital bones, so that the growth frequently causes protrusion of the eyeballs. There is always a generalized hyperplasia of the tissue involved, and aside from the invasive tendency there is no fundamental difference between chloroma and the usual forms of leucemia. Every transition from chloroma to ordinary myeloid leucemia has been described. There may be lacking any one or more of the three features peculiar to chloroma: the localized tumor masses, the green color of the tissue, and the invasive tendency of the growths with destruction of the substance of the bones. Cases of myeloid leucemia which are otherwise quite typical may have bone marrow that shows the green color on section.

The blood shows the changes characteristic of acute or subacute leucemia. The total leucocyte count may be but slightly elevated, or it may reach 500,000 or more. There are always immature cells present, commonly myeloblasts, usually in large numbers. The so-called chloroma cells are pathological myeloblasts. The disease progresses

steadily to a fatal termination within a few months, like the usual types of acute leucemia, quite uninfluenced by treatment. The symptoms differ from those of the commoner types of leucemia only in the pains, paralyses, or other local symptoms that may be caused mechanically by pressure from the growths.

Eosinophilic Leucemia and *Mast Cell Leucemia* are terms sometimes applied to cases of chronic myeloid leucemia which show a large percentage of one or the other of these cells. They do not form distinct types of leucemia, but are merely variants of the ordinary disease. The predominance of these cells may be merely transient features of these cases.

Plasma Cell Leucemia is a minor variety of lymphoid leucemia, usually acute, in which a large number of lymphocytes are of the plasma cell type.

Monocytic Leucemia is a term that has been applied to certain rare cases of leucemia in which at first large numbers of monocytes appeared in the blood. These cases have run the course of an acute myeloid leucemia, with the appearance later of many myeloblasts in the blood.

Pseudoleucemia is a term that was applied loosely to any condition that superficially resembled chronic lymphatic leucemia in showing a general glandular enlargement and an enlarged spleen, but which did not show leucemic blood changes. It has also been applied to cases of leucemia in an aleucemic stage. The term should be dropped.

Leucanemia is another misleading term that was

applied to certain cases that offered difficulties in differential diagnosis between pernicious anemia and myeloid leucemia, under the mistaken assumption that they represented a transition between the two diseases. Some of the cases were leucemias with a low total leucocyte count, and with a severe anemia associated with normoblasts and megaloblasts. Some were pernicious anemia with a "stimulation myelocytosis" during a normoblastic crisis. True transitions or combination forms of the two diseases do not exist, although some cases at times may present real difficulties in diagnosis.

Aleukia is a term that has been applied to a group of cases characterized by the clinical signs of extreme generalized aplasia of the bone marrow. There is severe anemia without evidence of regeneration of red cells, a leucopenia with great reduction in the granular leucocytes, and a marked reduction in platelets with a hemorrhagic diathesis. The cases run an acute, rapidly fatal course, uninfluenced by treatment. Aleukia is probably a clinical syndrome and not a disease entity. It may be met with, probably in some cases of acute aplastic anemia, of purpura hemorrhagica, and of acute (leucopenic) leucemia. These three diseases have features in common, and until more is known as to the etiology it will be difficult or impossible to classify all such cases precisely.

Lymphoblastoma is a term that has been applied to a group of diseases characterized by an abnormal hyperplasia of the lymphoid tissue, resulting in a more or less generalized enlargement of the

lymph glands and spleen. It does not include the infectious granulomata. It includes lymphatic leucemia, aleucemic lymphatic leucemia, lymphosarcoma, and (according to most of those who have used the term) Hodgkin's disease. The latter condition cannot logically be included, since it is the reticulo-endothelial cells and not the lymphocytes which undergo hyperplasia. The term has often been used merely to conceal an inability to make a more precise diagnosis.

Myeloma is a malignant neoplasm rising from any one of the several types of cells constituting the marrow parenchyma. Thus cases have been described arising from myelocytes, myeloblasts (the commoner type), lymphocytes, plasma cells, and erythroblasts. Tumors arising from endothelium and ordinary connective tissue, such as spindle cell sarcoma, and metastases from distant tumors, are not included. Multiple nodules develop, often widely distributed in many bones, but it is not a truly generalized diffuse process like leucemia. The symptoms are mainly (1) weakness, emaciation, and anemia, usual with any form of malignant neoplasm; (2) pains in the bones, spontaneous fractures, or rarely palpable nodules at the site of the growths; and (3) severe pains or paralyses variously located, due to pressure of the nodules on the spinal cord or nerve roots. The bones of the trunk are most involved. In the late stages there may be metastases to other organs. Death follows in from 6 to 18 months.

X-ray examination shows areas of erosion in the bones. The urine in the majority of cases contains

the *Bence-Jones protein.* On heating the urine a cloud appears when the temperature of 60° C. is reached, which dissolves on boiling to reappear on cooling. This protein is not pathognomonic of the disease, as it occurs occasionally in other conditions, including some cases of chronic myeloid leucemia, and metastatic carcinoma of the bone marrow.

The *blood* is never leucemic. The tumor cells rarely, if ever, get into the circulation. There may be a stimulation myelocytosis, with normoblasts, such as occurs in metastatic carcinoma of the bone marrow. In the end stages when marrow involvement is extensive, there may be a severe anemia of an aplastic type (myelophthisic anemia).

Agranulocytic Angina is a clinical and hematological syndrome of unknown cause characterized by an acute onset with fever, ulcerative pharyngitis or stomatitis, an extreme neutrophilic leucopenia, and a rapidly progressive course to a fatal termination. A critical survey of the published case reports makes it seem probable that we are dealing with a group of diseases rather than with a single disease. However, a considerable number of the cases stand out as a fairly well-defined group by reason of their similarity. These "typical" cases have all been in women, practically without exception. The onset is abrupt, usually in previously healthy individuals, with fever, general malaise, and prostration. There are necrotic ulcerative lesions on the tonsils, adjacent portions of the pharynx, gums, or tongue, which show little evidence of an inflammatory reaction. Vincent's organisms have been demonstrated in

some of the cases but not in all. The regional lymph glands are often enlarged, as is the spleen. The liver may be enlarged, and jaundice is common. There is no bleeding and no purpura.[1]

The *blood* changes are highly characteristic. The red cells, hemoglobin, and platelets are normal. There is a rapidly progressive diminution in the number of granular leucocytes, which may practically disappear. There are usually no myelocytes or myeloblasts, or an occasional one may be found after careful search. The absolute number of lymphocytes is also reduced, so that the total leucocyte count usually falls below 2,000, and may be as low as 100. The general clinical picture suggests a fulminatingly acute infection, but blood cultures either are sterile or have yielded organisms which at most can only be secondary or terminal invaders.

The disease ends fatally after an average duration of 8 to 10 days, but death may occur within 48 hours. At autopsy one does not find the pyemic foci of infection common in ordinary forms of sepsis. There is a disappearance of myelocytes and granular leucocytes from the bone marrow.

Many *atypical cases* have also been reported, but until something is known as to the etiology it will be a matter of individual opinion as to which of these should be included in the group agranulocytic angina, and which should not. Some of these atypical cases have developed extensive sloughing lesions, like noma. In a few cases some myelocytes and myeloblasts have appeared in the blood, suggesting

[1] Kastlin, G. J., "Agranulocytic Angina," *Am. Jour. Med. Sci.*, 1927, clxxiii, 799.

an acute leucopenic leucemia. In a few cases there has been a diminution in platelets, with bleeding and a posthemorrhagic anemia. Some cases have run a more protracted course, of many weeks. A few isolated cases have recovered, and one or two of these have suffered a second fatal attack. Some cases have been reported in males, but they were somewhat atypical, at least in most instances. Some severe cases of Vincent's infection have developed a leucopenia, and thus closely resembled agranulocytic angina.

The disease is probably not a form of leucemia. It is still uncertain whether the leucopenia is a result of injury to the marrow by some infection, or whether the marrow is injured by some other unknown agency, and resistance to infection is thus reduced. It is probably not related in any way to infectious mononucleosis. Its possible relationship to Vincent's angina needs further study.

CHAPTER XX

HEMORRHAGIC DISEASES

BY *hemorrhagic disease* (or diathesis) is meant any condition in which there is a tendency to bleed excessively. This is a group term which includes a large number of different diseases many of which are entirely unrelated except in the prominence of the one symptom, bleeding. Hemorrhage from localized pathological lesions, such as typhoid ulcers or nasal telangiectases, should not be included in this category. The abnormal tendency may be manifested by unduly protracted bleeding from cuts or bruises, by oozing from mucous membranes, or by extravasations of blood into the skin and mucous membranes (purpura). The tendency to bleed may be dependent on disturbances of blood coagulation or on abnormalities of the smaller vessels, especially of the capillary endothelium. Only those conditions in which bleeding is due primarily to abnormalities in the blood itself fall within the scope of this work.

A clear insight into disturbances of blood coagulation necessitates an understanding of the process of normal coagulation. Despite intensive study this is still imperfectly understood. The literature on the subject is bewildering to the uninitiated reader because of the numerous conflicting theories, the confusing diversity of terms which have been ap-

plied to the same substance, and the difficulty, in many cases, in differentiating between hypothesis and established fact. Of the current theories that of Howell is the most satisfactory. Those interested in this subject are strongly advised to begin the study by reading his articles.[1] The following discussion is based largely on Howell's views, but for didactic reasons the subject is presented perhaps more dogmatically and simply than the facts strictly warrant.

NORMAL BLOOD COAGULATION

The conspicuous part of the phenomenon of blood coagulation is the conversion of the soluble blood protein, fibrinogen, into the insoluble fibrin. Fibrinogen is a peculiar type of protein quite distinct physically and chemically from the other blood proteins. It is formed in the liver, and is present in relatively constant quantities in normal blood (about 0.4 per cent). When normal blood clots outside the blood vessels the fibrin is precipitated in the form of minute needle-shaped crystals which form an irregular meshwork, and probably cohere with one another at their points of contact. They hold the red cells within their meshes, and bind fluid probably by a process of physicochemical absorption rather than by physical imprisonment. In a normal clot the fibrin meshwork quickly begins to contract, and squeeze out the serum.

The conversion of fibrinogen into fibrin is brought about by a third substance, *thrombin*, formerly

[1] Howell, W. H., "Coagulation of the Blood," *Harvey Lectures*, 1916-17, 272. Howell, W. H., and Holt, E., *Am. Jour. Physiol.*, 1918, xlvii, 328.

called "fibrin ferment." Thrombin has not been identified chemically, and is recognizable only by its capacity to cause clotting of fibrinogen solutions. It is probably not a ferment.

Thrombin does not exist in active form in the circulating blood, but an antecedent substance is present which can be converted into active thrombin under suitable conditions. To this substance the name *prothrombin* (thrombogen) has been given. Its chemical nature is not known. Prothrombin may be obtained by extraction from blood platelets and from the marrow, probably from its platelet tissue. No other source has been demonstrated.

For the conversion of prothrombin into thrombin two substances are necessary:

(1) Ionized calcium salts in suitable concentration.

(2) A substance derived from the juice of injured tissue cells, or from disintegrating leucocytes and platelets. There has been a great diversity of opinion as to how this substance acts, and a correspondingly large number of different terms has been applied to it. Howell at first called it "thromboplastic substance," but he has since shown that the active constituent is the lipoid (phosphatid) *cephalin.*

The part played by *calcium salts* is limited to causing or facilitating the conversion of prothrombin into thrombin, and for this change they are indispensable. Hence, coagulation may be prevented by the addition, to perfectly fresh blood, of a soluble oxalate which precipitates the calcium, or of a citrate which probably forms a calcium salt that is ionized

to a slight degree only. Calcium is not necessary for the conversion of fibrinogen into fibrin by thrombin. Hence, the addition of thrombin in the form of fresh serum may cause oxalated plasma to clot. There is an optimum concentration of calcium for prompt coagulation which is not far from that found in normal blood. An artificial increase in calcium above this level will not accelerate coagulation, and may retard it. The exact manner in which calcium acts in this reaction is not known.

In addition to these substances which facilitate or actively participate in the process of coagulation, consideration must be given to those which specifically inhibit coagulation. To such substances the term "antithrombin" has rather loosely been applied. Theoretically such substances might act in two different ways: (1) They might prevent the formation of fibrin by thrombin, possibly by combining specifically with the latter and "neutralizing" it. Such substances would be *antithrombins* in the strict sense. (2) They might prevent the conversion of prothrombin into thrombin, by combining with, or in some way antagonizing, the prothrombin. Such a substance would be an *antiprothrombin*. Both of these types of inhibitory substances have been demonstrated.

The classical example of an antithrombin is *hirudin*, the active substance of leech extract. A minute amount added to a solution of thrombin will destroy the capacity of the latter to cause clotting of fibrinogen solutions. Similar activity has been demonstrated repeatedly in normal serum and

plasma. The chemical nature of the substance is not known. It is believed to be formed in the liver.

A substance known as *heparin*, having the activity of an antiprothrombin has been obtained from the liver by Howell. Heparin is a relatively simple substance, a glycuronic acid compound, free from N and Ph. It is present normally in the blood. Heparin itself has no direct antithrombic action, but Howell believes that it takes a part in the formation or activation of antithrombin in the blood. These two substances, heparin (antiprothrombin) and antithrombin, probably serve the highly important function of preventing coagulation of the blood within the vessels, by preventing largely the formation of thrombin, and by neutralizing such traces of it as may be formed.

Cephalin, the active substance in tissue juice which assists coagulation, is a direct antagonist both of heparin and antithrombin. In the test tube it abolishes the inhibitory action of these substances and brings about prompt coagulation. Howell believes that in normal coagulation cephalin acts by neutralizing heparin and antithrombin. Prothrombin can then be converted into thrombin by the calcium salts, and the thrombin formed is free to convert the fibrinogen into fibrin.

Ordinary clot formation, as seen in vitro, never occurs within the vessel of the living body. The form of blood coagulation which is of practical importance in stopping hemorrhage is the process of *intravascular* clotting, that is, thrombus formation, which is different from coagulation in the test tube. In thrombus formation the platelets play the initial

and the essential rôle. The platelets adhere in large numbers to the areas of damaged endothelium and to each other, and gradually accumulate to form a mass which may partly or completely occlude the vessel. This orderly deposition of platelets gives the thrombus a definite structure which the ordinary extravascular clot does not possess. Fibrin is then precipitated out of the plasma permeating the mass of platelets, which is thus converted into a coherent mass (thrombus). Retraction of the fibrin serves to complete the process of occluding the vessel.

In *summary*, the following substances are essential for coagulation, and lack of any one of them might be expected to cause bleeding: (1) fibrinogen, (2) calcium salts, (3) prothrombin, (4) cephalin, and (5) blood platelets.

(1) Lack of fibrinogen has been observed in grave liver disease, such as advanced cirrhosis, and phosphorus and chloroform poisoning. It has also been reported in a few cases of unknown cause, which have been termed fibrinopenia, or pseudohemophilia. Clinically the coagulation time and bleeding time are prolonged. The clot, if any forms at all, is scanty and flimsy. The blood platelets are not significantly reduced. Positive recognition depends on actual determination of the fibrinogen content of the blood.

(2) Calcium salts are deficient, at least in available form, in cases of chronic obstructive jaundice.[2] The tendency to bleed appears as a rule only if the obstruction is marked, and after it has lasted 4 to

[2] Lee, R. I., and Vincent E., *Arch. Int. Med.*, 1915, xvi, 59.

6 weeks. The coagulation time is definitely prolonged, often to double the normal. That calcium deficiency exists may be shown by the fact that the addition of a little calcium chloride solution to the blood hastens coagulation. The intravenous injection of calcium salts, or the administration of large doses by mouth for several days, will usually shorten the coagulation time and lessen the tendency to bleed. In other conditions the administration of calcium may cause a slight shortening of the coagulation time, but it has little, if any, demonstrable effect upon bleeding.

(3) A deficiency in prothrombin has not been demonstrated conclusively as a cause of hemorrhage. It has been described (but probably erroneously) in hemophilia.

(4) A deficiency of cephalin in tissue juice has not been demonstrated. A slow liberation of cephalin from platelets is probably the cause of the delayed coagulation in hemophilia.

(5) Deficiency of blood platelets is the commonest cause of pathological hemorrhage. It occurs in the specific disease "idiopathic (essential) thrombopenic purpura" and in a variety of other diseases in which there is bone marrow insufficiency or aplasia. The great importance of the blood platelets in coagulation deserves special emphasis. They are indispensable for thrombus formation. They are the main, if not the sole, source of prothrombin, and are one source of cephalin. Finally, their presence is essential for the normal retraction of the clot, which is the mechanism which brings about

the closure of bleeding vessels and cessation of hemorrhage.

Theoretically, bleeding might occur if heparin or antithrombin were present in excess. This condition has not been demonstrated, although it has been suggested as a cause of the disturbance in hemophilia.

For practical purposes of clinical diagnosis it is quite unnecessary to measure all these factors. A study should include the bleeding time, the coagulation time, with the simple test of Lee and Vincent for calcium deficiency, a platelet count, and an observation as to the retractility of the clot. These tests can be made quickly and easily, and require a minimum of apparatus and experience. In occasional cases it may be desirable to determine the fibrinogen or the prothrombin time.

PURPURA

By *purpura* is meant the spontaneous extravasation of blood into or under the skin and mucous membranes. It is not a disease but a symptom common to many diseases of diverse character and etiology. In some types of purpura there are disturbances of coagulation, but not in all.

By *purpura hemorrhagica* is meant a condition characterized by bleeding from the mucous membranes and by purpuric eruptions, associated with a marked reduction in blood platelets and a disturbance in blood coagulation. Purpura hemorrhagica is not a single disease, but a clinical syndrome which may occur in a number of different diseases. A preferable synonym is *thrombopenic purpura.*

ESSENTIAL THROMBOPENIC PURPURA

This is a specific disease of unknown etiology characterized chiefly by the syndrome of purpura hemorrhagica described above. The disease is probably dependent on a constitutional abnormality of the bone marrow. In some cases there is a hereditary tendency.

The disease occurs in an acute form with relatively brief attacks of from 2 weeks' to 2 months' duration, and more commonly, as a chronic form, with continuous or recurring symptoms. The disease occurs, or begins, most often in children. The onset is usually abrupt with bleeding from one or more mucous membranes, often protracted and uncontrollable by local applications. The bleeding in most cases is in the form of an oozing from the nose or gums. It may be from the uterus, stomach, intestine, or genito-urinary passages. In 4 of Pratt's 50 cases fatal cerebral hemorrhage occurred.[3]

Cutaneous hemorrhages occur in most cases and are often profuse. They may appear as numerous minute areas 1 to 5 mm. in diameter, (petechiæ), as larger areas 1 to 2 cm. in diameter (ecchymoses), or as large areas several inches in diameter (sugillations). They are usually most numerous over the limbs, especially the extensor surfaces, but are often numerous over the face and trunk. While the hemorrhages often occur spontaneously, a characteristic feature is the appearance of extensive areas of hemorrhage after slight trauma. The spots are al-

[3] Pratt, J. H., "Hemophilia and Purpura," Osler's *Modern Medicine*, Ed. 3, 1927, v, 101.

ways hemorrhagic, never erythematous, urticarial, or papular. The fresh spots are bright red, but it is impossible to make them blanch by pressure with a glass slide as in simple erythema. Older spots show the usual succession of color changes through dusky red, through bluish and blackish to brown.

Bleeding rarely occurs into joints or serous cavities. There is no fever, no nephritis, no endocarditis, no abdominal colic, no inflammation of the bleeding surfaces, and no arthritis, although pains in the joints are not uncommon (Pratt).

On physical examination little is found except signs of bleeding and anemia. The spleen is not enlarged.

The *Blood* during periods of bleeding uniformly shows an outspoken *reduction in blood platelets* which is usually marked (to less than 60,000), and in severe cases may be extreme (less than 10,000). However, while a significant reduction in platelets is invariable, there is not always an exact parallelism between the degree of reduction in platelets and the severity of the bleeding. The individual platelets are often abnormal, giant forms are common, or, particularly in the chronic cases, many of the platelets may be minute, so that the platelet count does not adequately reflect the degree of reduction in circulating platelet material.

The blood shows evidences of a posthemorrhagic secondary anemia which may become extreme. As a rule there are evidences of red cell regeneration, polychromatophilic red cells, occasionally a normoblast, and a neutrophilic leucocytosis. In other

cases there are evidences of a general bone marrow inactivity, with few if any immature red cells, and a leucopenia (see Aleukia).

The coagulation time in vitro is normal or only slightly and insignificantly prolonged. The clot that forms, however, is distinctly abnormal in its failure to retract in the usual manner. Slight degrees of nonretractility may be shown by the escape from the clot of abnormally large numbers of red cells. This phenomenon of nonretractility is due to the lack of blood platelets, and is quantitatively proportional to the degree of their diminution. To demonstrate that the failure to retract is significant, the clot must be detached from the walls of the test tube, and examined again after some hours as the clot from perfectly normal blood may adhere to the tube in such a way as to prevent retraction, until it is detached.

The *bleeding time* is greatly prolonged, as was pointed out by Duke. This is also directly proportional to the degree of platelet reduction. The time may be prolonged from the normal 2 to 3 minutes to 20 minutes, an hour or more.

The Rumpel-Leeds test shows a *diminished capillary resistance*. To make this test a blood pressure cuff is applied about the arm at the mean blood pressure for 5 minutes. The test is positive if within a few minutes after removal of the cuff a crop of purpura spots appears on the arm below the cuff. While a few spots may appear after the test in other conditions, a profuse crop of spots, and spots of large size (1 cm. in diameter) are almost pathognomonic of a thrombopenic purpura. This

phenomenon is also roughly proportional to the degree of platelet reduction.

The *course of the disease* varies greatly. Death may occur from hemorrhage within a few weeks, or after many months or years. The initial attack usually subsides after 1 to 3 months, and there may never be a recurrence of the bleeding. In most cases, however, the disease persists, and the symptoms run a remittent or intermittent course. A remission is always associated with a rise in platelets. This may occur quite abruptly. Within 24 hours the platelets may rise from a low figure to more than 100,000, with prompt cessation of bleeding. More often the rise is gradual. A remission may last for a few weeks only, or for many years. During these intervals there is often a definite reduction in platelets, but not to the point of causing bleeding. In some of the chronic cases the remissions are incomplete, the platelet count remains in the danger zone for bleeding (50,000 to 75,000), and slight bleeding persists. In either form an acute exacerbation may occur at any time.

Purpuric eruptions are absent in some cases. For a time bleeding may be limited to a single source, as the nose or the uterus. Uterine bleeding, if dangerously profuse, may be stopped by hysterectomy, or sterilization with radium, but if this be done bleeding is apt to recur sooner or later from some other source.

The *differential diagnosis* depends on the demonstration of the characteristic blood changes (diminished platelets, prolonged bleeding time, normal coagulation time, nonretractile clot), on the

exclusion of other types of purpura, and of diseases that might cause a symptomatic thrombopenic purpura. Among the latter may be mentioned:

(1) Severe intoxications (benzol, arsphenamine).

(2) Severe infections (sepsis, typhoid, diphtheria).

(3) Anaphylactic shock.

(4) Aplastic anemia.

(5) Pernicious anemia in some stages.

(6) Acute leucemia.

There remains a large miscellaneous group of conditions in which purpuric eruptions occur, but in which the platelets are normal in number, hemorrhages are usually insignificant or absent, and the purpura presumably is dependent on changes in the capillary endothelium. Among these are:

(1) Intoxications, as with iodine, arsphenamine, snake venom, and in many forms of drug idiosyncrasies.

(2) Acute infections, such as sepsis, meningitis, typhus fever, smallpox, and especially bacterial endocarditis, in which infective capillary emboli occur.

(3) Senile or cachectic purpura, chiefly on the dependent portions of the body, due to local vascular weakness.

(4) Scurvy. In this disease the gums are swollen and spongy but they do not bleed, nor do the other mucous membranes. Large hematomata develop under the periosteum, or in the deep muscles of the thigh and calf. The eruption consists of a few small purpuric spots around the hair follicles, chiefly of the lower legs, and they are fewer and less generalized than in purpura hemorrhagica.

(5) Anaphylactoid purpura includes a heterogeneous group of cases, occurring chiefly in children and young adults, which present one or more of the following groups of symptoms:

(a) Cutaneous eruptions, usually with erythema, urticaria, and angioneurotic edema as well as purpura (Erythema multiforme). Many cases of "purpura simplex" probably belong in this group, cases in which a few purpuric spots constitute the only sign of illness.

(b) A mild arthritis, with pain, tenderness, and slight swelling of the joints, especially of the knees and ankles (Schoenlein's purpura, peliosis rheumatica). Purpuric spots may be clustered about the joints involved.

(c) Attacks of acute, colicky abdominal pain, often severe and associated with abdominal tenderness and rigidity. There is frequently blood in the stools and vomitus, though the bleeding is never profuse. This syndrome is usually met with in children, and is generally associated with cutaneous eruptions. It is commonly known as Henoch's purpura.

These symptoms appear in recurring rather brief acute attacks, with fever, and more or less prostration and general malaise. Acute hemorrhagic nephritis may occur, chiefly in cases that show intestinal colic, but it is relatively uncommon. Recovery is the rule, but death may occur from uremia, or a chronic nephritis may follow. These patients often give violent reactions to tuberculin, or to injections of other foreign protein.

The platelet count, the bleeding time, coagulation time, and retractility of the clot are normal, and the capillary resistance test is negative. There is no anemia. There may be a slight leucocytosis.

The etiology of this group of cases is obscure. It has been suggested that the symptoms are due to an intoxication with a histamine-like substance which makes the capillary walls abnormally permeable to the constituents of the blood. There are many points of resemblance to serum disease, but the resemblance to purpura hemorrhagica is only superficial. Differentiation from thrombopenic purpura is practically important, because the treatment is entirely different. In anaphylactoid purpura transfusions are unnecessary, and splenectomy is useless and inadvisable. Calcium salts may be given by mouth or by intravenous injection with benefit in some cases. Patients usually recover promptly from an attack of anaphylactoid purpura, and are perfectly well in the intervals, but recurrences are the rule.

Hemophilia must be differentiated from purpura by the history and by a study of the coagulation factors. The differences are discussed under hemophilia, page 236.

Treatment. The only effective method of stopping bleeding promptly in severe cases of thrombopenic purpura is *transfusion.* The relief lasts only 3 or 4 days, which is the probable average life of the platelets introduced. Transfusion must then be repeated. In this way bleeding may usually be controlled until the hemorrhagic tend-

ency spontaneously subsides. In this condition transfusion of unaltered blood sometimes seems to be more effectual than citrated blood, and in some chronic forms of purpura repeated small transfusions of this type seem to have a distinctly favorable lasting effect.

Rest in bed is essential and may suffice to stop bleeding in the milder cases. Exposure of the skin to sunlight or ultra-violet radiation, or life in a subtropical climate, seems to benefit some patients.

Calcium salts, serum, or thromboplastic preparations, however administered, usually have little or no effect.

The most dependable means of obtaining sustained relief is *splenectomy*.[4] In almost all cases bleeding stops promptly, and there is usually an immediate and marked rise in platelets. After some weeks or months the platelet count again falls, but in most cases bleeding does not recur. In a few cases, however, it has recurred. While it is not yet possible to state the duration of the remissions thus obtained, in most cases they have been well maintained, and have amounted to a symptomatic cure. Splenectomy is to be recommended in practically all cases in which bleeding is persistent and incapacitating. It is not certain just how removal of the spleen stops bleeding. It is possible that the spleen has been actively destroying platelets, or that it has been exercising an inhibiting influence over platelet formation in the marrow.

[4] Giffin, H. Z., and Halloway, J. K., *Am. Jour. Med. Sci.*, 1925, clxx, 186.

HEMOPHILIA

Hemophilia is a rare hereditary disease characterized by a tendency to bleed protractedly from slight injuries, and by a marked prolongation of the coagulation time of the blood.

The disease is dependent on a constitutional peculiarity of the individual. There is no external cause. The disease is (practically) limited to males, and is, in general, transmitted solely through females, the "conductors," who themselves show no symptoms of bleeding. It is transmitted as a sex-linked recessive Mendelian characteristic. The tendency may be transmitted through many successive generations of females, to reappear in some remote male descendant. On the average half the daughters of a conductor will be conductors, and half her sons will have the disease. While in general the statements are true that only males have the disease and only females transmit it, geneticists have pointed out that with this type of hereditary transmission, one would expect half the daughters of a hemophilic male to become conductors, and be able to transmit the disease to their sons. Fairly well-authenticated cases of this transmission have been reported. It would also be possible, theoretically, for a female to have the disease if she sprang from the union of a hemophilic male and a female conductor. All the sons of such a union should theoretically have the disease. In view of the rarity of the disease the chance of this happening accidentally is very small, and no authentic case of hemophilia in a female is on record.

The condition exists from birth, and bleeding always occurs during infancy and childhood. The severe cases die in childhood, often during the first year. Bleeding which begins at a later age is not due to hemophilia.

The characteristic clinical feature is the tendency to bleed protractedly from small cuts and bruises which would stop bleeding promptly in a normal individual. It is not so much the occurrence of bleeding, or the rapidity of the bleeding at a given moment, as the duration which is pathological. Fatal bleeding has often followed simple operations, such as tooth extraction or circumcision. However, profuse bleeding is more apt to occur from ragged cuts and scratches than from clean incisions. The bleeding persists for hours, and often for days and even weeks, as a slow oozing that eventually may be exsanguinating. "The blood drips from a wound as it would from a blood soaked sponge" (Pratt). A simple pin prick does not bleed abnormally, and one can safely puncture a vein or the finger or ear for purposes of examination. The bleeding time is usually normal (Duke's method).

Hemorrhages from the mucous membranes of the nose and gums may occur, but bleeding from other mucous membranes is uncommon.

Subcutaneous hematomata are common. They follow traumata, but in the severer cases the mere pressure of the clothes on the skin may be sufficient to start one. They may be relatively small black and blue spots, or they may become enormous extravasations, covering as much as half of the entire chest wall. Death has occurred from such "inter-

nal" hemorrhages. They may cause intense pain, or paralysis from pressure on the nerve trunks. There may be fever and leucocytosis. The areas may resemble phlegmons, and have been incised with disastrous results. Purpuric eruptions of the ordinary type do not occur.

Hemorrhage into the joints is also common. The knees are most often attacked, next in frequency the other large joints of the limbs, less often the small joints of the hands. The symptoms are swelling of the joint, pain, and disability. The degree of swelling may be trivial, and it may subside within a day or two, or it may be enormous and last for weeks. There may be fever and leucocytosis, simulating an infectious arthritis. At first the blood may be entirely absorbed, leaving the joints quite normal, but sooner or later there comes a hemorrhage which is not so well absorbed. Organization of the remaining blood occurs, adhesions form within the joint, and partial disability or occasionally ankylosis follows. As a rule, if the joints are once involved, large numbers of attacks occur.

Aside from the hemorrhages there are no notable symptoms other than those which are due to anemia. On physical examination the patient shows nothing significant except evidence of hemorrhage and anemia. The spleen is not enlarged. The appearance of the affected joints on X-ray examination is fairly characteristic.

The *blood* cells are normal morphologically, or show merely the changes of a posthemorrhagic secondary anemia. The blood platelets are normal in

number (or increased after bleeding), a very important diagnostic point. A study of the blood coagulation factors shows a characteristic picture. The bleeding time is normal or practically so. The blood clot after it once forms retracts normally. The capillary resistance test is normal. The coagulation time in vitro, tested with blood obtained by venapuncture, is greatly prolonged. Instead of the normal of 6 to 15 minutes, it is often one hour, and may be several hours. However, coagulation does eventually occur. The prothrombin time (Howell's method) is similarly prolonged.

In some cases, during intervals of relative remission, the retardation of coagulation is less marked, and repeated tests on different occasions may be required to show a significant abnormality. A slight delay (to 20 or 25 minutes) is not diagnostic, as it is seen not infrequently in other conditions. Methods of testing coagulation time by the use of finger blood also show a delayed coagulation, but these methods are less dependable than those which use venous blood.

There is still uncertainty as to the pathogenesis of the coagulation defect. Many conflicting theories have been advanced. The weight of evidence now favors the view that the disturbance is due to a qualitative defect in the platelets. They are mechanically more resistant than those of normal blood, and they act more slowly and less effectively in initiating the clotting of hemophilic plasma. Howell has suggested that a slow disintegration of the platelets and a correspondingly slow

liberation of cephalin may be immediate cause of the delayed coagulation.[5]

Diagnosis. The typical coagulation disturbance, in conjunction with the family history, the early age of onset, and the recurring attacks of bleeding of the type described suffice to make the diagnosis. The disease is not apt to be overlooked. More often the diagnosis is made erroneously. The commonest error is to make the diagnosis of hemophilia because of a single profuse bleeding. In an adult this is always an error. Bleeding in hemophilia is a chronic recurring condition, beginning in infancy. This at once eliminates from consideration a great many cases of bleeding.

Differential diagnosis. The chief difficulty is in differentiating hemophilia from certain cases of chronic purpura hemorrhagica, particularly the group with a hereditary history. In purpura both males and females are attacked, and in the hereditary cases either parent may transmit the disease. In most cases a blood examination is decisive. In hemophilia there is a normal platelet count, a normal bleeding time, and a prolonged coagulation time; in purpura, a low platelet count, a prolonged bleeding time, and a normal coagulation time. In some cases these characteristics are not always pronounced, and a diagnosis may be impossible on a single examination.

Hemophilia in extremely young infants might be confused with the more common hemorrhage

[5] Howell, W. H., and Cecada, E. B., *Am. Jour. Physiol.*, 1926, lxxviii, 500.

of the new-born. The latter condition may usually be recognized by the bleeding from the umbilicus, and by the melena, and by the speedy and permanent recovery.

Prognosis. The disease lasts throughout life and is incurable. The tendency to bleed varies greatly in intensity in different indivduals, and may vary in the same person from time to time. The cause of such fluctuations is not known. The outlook in general is unfavorable. A majority of the cases die in infancy or childhood. Death from the first hemorrhage is rare, but every hemorrhage is a source of danger. With care a minority of the milder cases reach adult life. In such cases there may be a tendency for the bleeding to lessen with advancing age. Repeated hemorrhages into the joints usually result in considerable eventual deformity and disability.

Treatment is purely preventive and palliative. The utmost care must be taken to avoid injuries, and children must be kept out of rough games and sports. No operation of the most trivial sort, and in particular no tooth extraction, should be undertaken without preliminary transfusion. Residence in warm climate is desirable. Cephalin may be administered by mouth over long periods, but the available evidence indicates that it is of little value.

To stop bleeding local measures should be tried; packing or the application of a pressure bandage if practicable, and local application of hemostatic substances. Of these cephalin is the best. Cephalin is a rather unstable substance and is best used in the

form of impregnated gauze put up in vacuum sealed tubes. If this is not available, other thromboplastic preparations may be tried, or gauze soaked in normal fresh serum or whole blood (human or animal). In general epinephrin solutions and simple styptics are useless, and the effect of any local application is uncertain.

Transfusion is the only measure which is reasonably dependable. Either unaltered blood or citrated blood may be used, provided the platelets are not removed. Small amounts of blood may suffice, but not with the certainty of large transfusions (500 cc.). Bleeding practically always ceases promptly, and there is a corresponding shortening of the coagulation time. The effect of a transfusion lasts only 2 to 4 days, approximately the life of the platelets introduced. Another transfusion must then be given unless the injury has healed in the interval. Theoretically the number of transfusions is limited only by the number of available donors. Practically transfusion will usually be limited to patients who have wounds of appreciable gravity, who require operation, or who bleed alarmingly despite the use of local measures. The tendency in all cases is for the bleeding to stop, and for any single attack of bleeding the probability is that it will stop.

If transfusion is not available, intravenous injections of fresh normal serum (preferably human) should be tried, or intramuscular injections of fresh normal serum, whole blood, or of some thromboplastic preparation. None of these even remotely compares with transfusion in efficacy.

As a matter of precaution every hemophilic should have one or more compatible donors available in case of emergency. He should also carry cephalin gauze as a local application for cuts and bruises.

HEMORRHAGIC DISEASE OF THE NEW-BORN

This is a self-limited disease occurring in infants during the first one or two weeks of life, characterized by a marked tendency to bleed, especially from the umbilicus and intestine (melena neonatorum).

The *etiology* is unknown. The disease is not inherited. A minority of the cases have been associated with congenital syphilis or other infections. Robust healthy-looking infants may be attacked. The disease is not rare in hospital practice (0.5 to 1. per cent of all births). Both sexes are equally involved.

Symptoms. The onset is usually on the second, third, or fourth day, with spontaneous bleeding, appearing as a persistent oozing, usually from many areas. There is no tendency to clot, and the infant often becomes exsanguinated. Especially frequent are:

(1) Cutaneous hemorrhages, either oozing from abrasions, ecchymoses, or large hematomata, especially of the scalp.

(2) Bleeding from the umbilicus.

(3) Melena, usually appearing as copious tarry black stools, less often as bright fresh blood. A profuse, even fatal, hemorrhage into the intestine

may occur without the immediate passage of blood. The black tarry stools in this disease may be mistaken for normal meconium, and if there is the slightest suspicion of bleeding the stools should be examined chemically for occult blood.

(4) Intracranial hemorrhages, which are often fatal. Especial attention should be paid to the presence of drowsiness, convulsions, paralyses, or signs of increased intracranial pressure, as evidences of intracranial hemorrhage.

Bleeding may occur from the eye, nose, mouth, stomach, or genito-urinary tract.

The *blood* cells show no characteristic abnormality. The usual phenomena of a posthemorrhagic anemia appear. The platelets have been reported in some cases to be normal in number. The study of the coagulation factors has been scanty and the results conflicting. More data are urgently needed to determine the mechanism of the disturbance.

The *mortality* in untreated cases is from 60 to 80 per cent. Death usually occurs within 3 days. If the infant survives complete and permanent recovery follows. While bleeding may continue for two weeks, it usually ceases within a few days.

Diagnosis. A normal infant may vomit a little blood that was swallowed from its mother's nipple, or there may be slight vaginal bleeding. Otherwise any bleeding in an infant should receive serious consideration. The more important conditions to differentiate are hemophilia and thrombopenic purpura. Bleeding due to these diseases is rare during the first two weeks of life. In hemophilia

bleeding from the umbilicus is rare, and the melena is relatively uncommon. The family history is usually decisive. The great reduction in platelets in purpura will generally differentiate it from hemorrhagic disease of the new-born.

Treatment. Transfusion should be given as soon as the diagnosis is made, even in the mild cases. Any case may become severe, and the risk of a crippling intracranial hemorrhage should not be incurred. Citrated blood is perfectly satisfactory. It may be given into the superior longitudinal sinus or the external jugular vein if the median basilic vein is too small. From 30 cc. to 125 cc. should be given, according to the degree of the anemia. If the condition is urgent either parent may be used as a donor without preliminary tests for compatibility, since agglutinins are as a rule not developed in young infants. Bleeding practically always stops and a second transfusion is rarely necessary. The mortality in Vincent's series of treated cases was 12 per cent,[6] and early treatment should reduce this figure practically to zero.

If there are signs of intracranial hemorrhage, a decompression should be done promptly after transfusion. If transfusions are impossible, some benefit may be obtained by repeated intramuscular injections of fresh human serum or whole blood.

MISCELLANEOUS HEMORRHAGIC DISEASES

Not all cases of hemorrhagic diseases will fit into the categories that have been described here. A

[6] Vincent, B., Nelson's *L.L.L. Med.*, 1920, iv, 146.

number of such cases has been reported, which may be examples of new clinical syndromes. It is as yet impossible to classify these cases, and a detailed discussion of them is beyond the scope of this book.

CHAPTER XXI

TRANSFUSION

THE great importance of transfusion as a therapeutic procedure in diseases of the blood necessitates a consideration of the subject in this book. The idea of transfusion is old. Transfusion of patients with blood from animals was tried more than two centuries ago. Blood from a foreign species is rapidly destroyed after transfusion, and usually causes violent reactions, and this procedure was quickly given up. Transfusion of human blood was practiced quite extensively from fifty to seventy-five years ago, but the violent reactions that occasionally followed transfusions for a long time overshadowed the benefit that might otherwise have been obtained from them. Transfusions could not come into general use until it was shown that these reactions were due to hemolysis of the red blood cells introduced, and could be foreseen and avoided by a proper selection of donors. The selection of a suitable donor is the crucial step in the safe performance of transfusion.

The donor must be a healthy individual, preferably a young adult, free from infectious diseases. The most important of these is syphilis, and the danger of transmission from donor to recipient is great in the primary and secondary stages of the dis-

ease. The risk of transmission is probably small in the late tertiary and latent cases. A negative Wassermann reaction is essential but is not sufficient. An explicit history must be taken and a complete physical examination of the donor must be made. The writer has seen syphilis transmitted by a donor with a negative Wassermann reaction who was found later to have had a primary lesion. Malaria has also been transmitted accidentally by transfusion.

The blood of the donor must be compatible with that of the recipient. Blood which is incompatible is hemolyzed and destroyed like blood from a different species of animal in the body of the recipient, who suffers a violent and sometimes fatal

DIAGRAM

SHOWING THE RELATIONSHIP OF THE CELLS AND SERA OF THE DIFFERENT BLOOD GROUPS

	Cells AB	Cells A	Cells B	Cells O	Cell Receptors	Serum Agglutinin	Moss Group	Jansky Group	Frequency
Serum AB	o	o	o	o	AB	o	1	4	10%
Serum A	+	o	+	o	A	β	2	2	40%
Serum B	+	+	o	o	B	α	3	3	10%
Serum O	+	+	+	o	O	$\alpha\beta$	4	1	40%

Agglutination is indicated by +, and the absence of agglutination by o.

reaction as a result. The compatibility of a prospective donor can be determined by suitable tests for the presence of isoagglutinins in the bloods in question.

It has been shown that (at least for the most part) all human beings fall sharply into one of four different groups or types, depending on the capacity of their serum to agglutinate the red cells of other individuals and on the susceptibility of their cells to agglutination by other human serum. The interrelationships that have been shown to exist between the cells and serum of these different groups can be shown most simply by means of a diagram. Hemolysis sometimes occurs in vitro in association with agglutination, but never in its absence.

As is illustrated in the diagram, the cells of any individual are never agglutinated by the serum of another individual belonging to the same group. Those individuals classified as Group O possess cells which are not agglutinable in any (human) serum, while cells from Group AB individuals are agglutinated by the serum of all other groups. Conversely, the serum of Group O individuals will agglutinate cells from the other three groups, while serum from Group AB has no agglutinating activity. Groups A and B are reciprocals of each other, cross-agglutinating each other's cells, while the serum of both agglutinates Group AB cells, and the cells of both are agglutinated by Group O serum.

The capacity of a serum to agglutinate cells may be referred to a hypothetical active substance in the serum which is known as an agglutinin, or iso-

agglutinin, the prefix "iso-" indicating that the action is exerted on cells of other individuals of the same species. The capacity of the cells to be agglutinated may similarly be referred to some special property (or part) of the cells, specifically corresponding to the agglutinin that acts on it, which has been termed an agglutinogen or, more simply, a "receptor." The use of the term "receptor" is to a considerable extent justified by the fact that the cells actually combine with the agglutinin and remove it from the serum during the process of agglutination.

The observed facts, recorded in the diagram, may be explained on the following hypothesis: There may occur in human serum two different isoagglutinins which are designated arbitrarily as Agglutinin α and Agglutinin β. There may occur in human cells two different receptors, designated as Receptor A and Receptor B, which correspond specifically to the two agglutinins. In a given serum there may be no agglutinin, or either one, or both of them may be present. Similarly, the red cells of a given individual may possess no receptors, or one or both receptors may be present. However, one never finds an agglutinin and its corresponding receptor present in the same blood. Such an occurrence presumably would be incompatible with life. The distribution of agglutinins and receptors which is believed to exist in the four groups is also shown in the diagram. In any given combination of cells and serum agglutination occurs whenever an agglutinin and its corresponding receptor are both present. Strong confirmatory evidence of the cor-

rectness of this hypothesis has been obtained by agglutinin absorption tests.

The behavior of the cells (possession of receptors) is the fundamental characteristic which determines the group of the individual. This is a constitutional peculiarity which is inherited as a dominant Mendelian characteristic. Agglutinins (and sometimes receptors) are usually not demonstrable at birth, but become evident after 3 months to a year. This explains why new-born infants can usually receive blood safely from any donor without preliminary tests. There is no evidence that the group ever changes after it is once established, and there is every reason to believe that any inherited characteristic of this sort is as fundamental and immutable a trait of the individual as is the color of his hair or the general configuration of his body. The agglutinating activity of the serum may fluctuate in intensity or even disappear, but this does not affect the group of the individual in the least. The same four groups have been found in all human races which have been studied. The relative frequency of the different groups in the Caucasian races of Europe and North America is indicated in the diagram. The frequency in certain other races is somewhat different from these figures.

Since the possession of receptors by the red cells is the factor which determines the blood group, the logical method of designating the groups is by the use of symbols which indicate the receptors present in each case, rather than by meaningless arbitrarily chosen numerals. This method will henceforth be followed here, and the groups will be designated as

Group AB (Group 1, Moss), Group A (2), Group B (3), and Group O (4).

The practical importance of this subject is due to the fact that the behavior of a mixture of cells and serum in the test tube is an indication of what their behavior will be in the body. It is found in practice that blood is compatible for transfusion if the serum of the recipient does not visibly affect the cells of the prospective donor in preliminary tests in vitro. However, if in such a mixture the cells are agglutinated or hemolyzed the blood is incompatible, and if used for transfusion the red cells introduced will be destroyed, and there will often be a violent reaction with hemoglobinemia, hemoglobinuria, and jaundice. While, theoretically, it is desirable that the donor and recipient belong to the same group, in practice this is not necessary. It is absolutely essential that the cells of the donor shall not be agglutinated in the serum of the recipient, but it is usually of no moment if the cells of the recipient happen to be agglutinable in the serum of the donor. The donor's serum is probably so much diluted in the blood of the recipient that its agglutinating and hemolytic activity is abolished. Therefore, it is, in general, safe and satisfactory to use an individual belonging to Group O (4 Moss) as a donor for a patient of any group. The use of blood from a Group O donor for a patient belonging to another group is no more likely to cause reactions than is blood from a member of the corresponding group. With certain limitations which will be discussed later, Group O individuals may be regarded as universal donors. Similarly, because

of the absence of iso-agglutinins from their serum, individuals belonging to Group AB may be regarded as universal recipients.

The first step in establishing the compatibility of a prospective donor is to determine the blood group of both the donor and the recipient. If both belong to the same group, the donor (with possibly rare exceptions) will be compatible. However, it is important also to test the action of the recipient's serum on the prospective donor's cells and insure that no agglutination occurs before proceeding with the transfusion. The reasons for this are (1) that this "cross-matching" serves as a very valuable check on the accuracy of the group determinations. Even with experience and care errors may otherwise creep in. And (2) while the great majority of individuals fall clearly into the four groups described by Jansky and by Moss,[1] cases have been reported which do not correspond perfectly to this classification (Guthrie and Huck).[2] Blood from such individuals might show cross-agglutination reactions which would not be anticipated by ordinary group determinations. In practice incompatibility due to this cause must be distinctly rare. Unexpected reactions after transfusion, if due to the use of incompatible blood, in the writer's experience can almost invariably be traced directly to technical errors in the preliminary tests.

The detailed technique for making these prelim-

[1] Moss, W. L., *Bulletin Johns Hopkins Hosp.*, 1910, xxi, 63.

[2] Guthrie, C. G., and Huck, J. G., *Bulletin Johns Hopkins Hosp.*, 1923, xxxiv, 37, 80, and 128.

inary tests is outlined in Chap. XXII, and the more serious sources of error are discussed in detail. For routine purposes it is not necessary to test the blood to be examined with serum and cells from all four groups. It is quite sufficient to test the cells of the patient with serum from Group A and from Group B, and as an additional check, preferably from Group O also.[3] This abbreviated method will not reveal such atypical individuals as Guthrie and Huck have described, but if the action of the recipient's serum on the donor's cells is also tested any reaction due to an unexpected incompatibility of this sort will be avoided.

Rouleaux formation has nothing whatever to do with agglutination, and if it can definitely be recognized as rouleaux it is of no practical significance. Rouleaux formation is no contraindication to the use of blood for transfusion. The writer has given transfusions of citrated blood in which the red cells were rouleauxed in maximal degree without any reaction whatsoever. However, rouleaux formation causes many errors in group determinations and is responsible for many of the cases of alleged change of group. In any specific instance in which it is uncertain whether agglutination or rouleaux is present, the patient should be given the benefit of the doubt and a different donor secured.

An individual should not be used a second time as donor for the same patient without repeating the preliminary tests for compatibility, even though the first transfusion caused no reaction. In most cases repeated transfusions from the same donor are well

[3] Moss, W. L., *Jour. Am. Med. Assn.*, 1917, lxviii, 1905.

borne, but it has happened that a second transfusion was followed by a serious reaction. The writer is doubtful whether the usual preliminary tests are certain to reveal incompatibilities of this particular type, but no precaution should be neglected.

METHODS OF ADMINISTRATION

The two methods in common use are the "citrate" method and the "syringe-canula" method. In the latter method the blood is transferred directly from donor to patient by means of syringes and special canulas, without the use of any anticoagulant. One of the most widely used of these devices is the canula of Unger, and in the hands of one skilled in its use it is satisfactory. The advantage of the method is that theoretically the blood is less altered than after the addition of an anticoagulant, and that practically reactions (of the simple febrile type) are less frequent than after citrate transfusions. There are several disadvantages. It is much more of an ordeal for the patient, and necessitates bringing the donor and patient together. It is necessary, once the transfusion is started, to continue it with considerable speed, or blood will clot in the apparatus. It is not practicable to make the injection slowly, or to interrupt it temporarily, as is not infrequently desirable. It requires a considerable degree of skill and continued practice to insure carrying out transfusions with uniform success. One or two trained assistants are necessary. It is not a method which the average physician or surgeon without special training can undertake as an occasional procedure, and its

use must largely be restricted to the specialist. (For details of the method see Unger's publications.)

Because of its greater simplicity, the citrate method is the most practicable, and in many situations the only feasible method, and for that reason the technique will be discussed in more detail. Blood from the donor is drawn by gentle suction through a fairly large needle into a sterile (closed) flask, in which it is intimately mixed with one-tenth its volume of 2.5 per cent sodium citrate solution. It is important that the venapuncture be carried out cleanly, and that there be no admixture of tissue juice with the blood. It is also important that a continuous free flow of blood be maintained. Otherwise there will be a tendency for small clots to form in the tube and needle, and a febrile reaction may result. The flow is facilitated by alternate flexion and extension of the hand. The tourniquet must not be applied tightly enough to interfere with the radial pulse.

Unless an unusually large transfusion is to be given a slight excess of citrate does no harm and is preferable to a deficiency, but a gross excess is to be avoided. On the other hand, clotting is liable to occur if the concentration of citrate in the blood is less than 0.25 per cent. The quantity administered with an average transfusion of 500 cc. is only a quarter of the maximum safe dose (5 grams). Contrary to what might be expected the intravenous injection of sodium citrate in these doses does not retard coagulation, but actually accelerates it.

The blood should be given to the patient as soon as practicable after it has been drawn, though

the delay of an hour or two makes little if any difference. Blood which has been kept in the ice box for 24 hours or even longer has been used successfully, but such blood is more apt to cause febrile reactions and its use is not advised. The blood should be introduced slowly by gravity, from an infusion bottle or similar apparatus, and except perhaps for exsanguinated patients, or when a fine needle has to be used, the blood should not be forced in. It is the custom of the writer to introduce only about 50 cc. of blood during the first ten minutes. If through some error incompatible blood should be given, symptoms of a reaction will usually appear within this time, and the transfusion can be stopped before a dangerously large quantity of blood has been introduced. If no symptoms have appeared after 10 minutes, the remainder of the blood can be run in much more rapidly. The blood must be kept at body temperature while it is being injected. This can be done conveniently by coiling a loop of the rubber tubing through which the blood is flowing, in a basin of warm water.

It is desirable to filter the blood through a thin layer of sterile gauze as a precaution to avoid the injection of small clots. Should one or two small clots be present the blood may still be used, although the risk of a febrile reaction is somewhat increased. Should any considerable amount of clotting occur the blood should be discarded. Even though the clots be filtered out, the use of such blood is likely to cause severe reactions. This is not as dangerous as might be expected, however. Defibrinated blood was used for transfusions for a

time with limited success before safer practicable methods were available. Filtering the blood in this way undoubtedly removes some platelets, and in the thrombopenic purpuras and hemophilia the blood should not be filtered.

The citrate solution and the salt solution which is used to flush the apparatus and fill the tubing should be made from chemically pure materials and freshly distilled water, and should be filtered and sterilized at once. The solutions should be used within 24 or 48 hours of their preparation. The apparatus and particularly the tubing must be kept scrupulously clean and free from clotted blood. If new rubber tubing is to be used, it must be boiled in 1 per cent sodium carbonate solution and washed out thoroughly before use. Neglect of any of these precautions will increase the liability to reactions. The febrile reactions which occur especially after citrate transfusions are rarely serious or dangerous. The frequency and severity of the reactions diminish as the skill and experience of the operator increases, and with adequate care they can be reduced to a relatively low figure. The red cells introduced in citrated blood are just as effective as in unaltered blood, whatever be the type of anemia present.

DANGERS OF TRANSFUSION

(1) The danger of infection from the donor has been discussed. With reasonable care to maintain asepsis, infection by contamination from outside sources practically never occurs.

(2) Dilatation of the heart or acute pulmonary edema may occur if the blood volume is increased too abruptly. This rarely happens unless there is preëxisting cardiac disease, or unless an excessive amount of blood is injected. Except after acute hemorrhage the volume of blood injected should not exceed 500 cc., or at most 600 cc. The blood should be injected slowly and with especial care if myocardial weakness exists. Warning signs are a rising pulse rate, a falling blood pressure, a feeble pulse of small volume, and a vigorous dry cough, persistently repeated. Later there may be dyspnea, oppression, and a productive cough (pulmonary edema).

(3) Reactions to the blood introduced.

The simple febrile reaction is the commonest type. A rise in temperature of ½ to 1 degree without other symptoms is fairly frequent. Definite symptoms of reaction occur in from 10 to 20 per cent of the cases, but should not be disturbing in more than half this number. They are less frequent after the skillful administration of unaltered blood, but may occur with any method. The reaction usually begins about one hour after the transfusion is started, although it may be delayed for 24 hours. There may be a chill, sometimes quite violent, with a rapid rise in temperature of 2 to 6 degrees, with headache, discomfort, nausea and vomiting, tachycardia and prostration. The temperature reaches its height within an hour or two, and rapidly falls to normal again. While such reactions are disagreeable if severe, they are rarely of serious consequence. The gain in hemo-

globin and in red cells after such a reaction on the average is just as great as in cases where no reaction occurs.

The cause of these reactions is not known. There is great variation in the tendency of different individuals to react in this way. These reactions have no relation to agglutination or hemolysis, and are not followed by hemoglobinuria or jaundice. They cannot be anticipated by preliminary blood tests. There is probably more than one cause involved. Among the factors which have been held responsible are (1) errors in technique, particularly in preparing the solutions; (2) the liberation of toxic substances probably from the platelets during the incipient stages of blood coagulation; and (3) injury to the red cells by the citrate. The writer does not believe that the latter factor is of any practical significance. The majority of these reactions resemble closely the reactions that may follow the intravenous injection of glucose or physiological salt solution, and the mechanism, whatever it may be, is probably similar.

Febrile reactions rarely require anything more than acetyl salicylic acid or codein to relieve discomfort. Epinephrin should be given promptly if the reaction is severe, or if there is any doubt as to the character of the reaction.

The hemolytic reactions which follow the injection of incompatible blood constitute the most serious type. They can be foreseen and avoided by adequate preliminary tests and, therefore, are extremely rare in the hands of careful experienced

workers. They are just as likely to occur after "direct" as after citrate transfusions.

The symptoms of such reactions are quite characteristic. They usually begin quickly, severe ones within ten minutes after the first blood is injected. They may begin after as little as 20 cc. of blood has been given, and if the transfusion is given slowly will usually begin before 100 cc. has been exceeded. The early symptoms are restlessness, anxiety, generalized painful, tingling sensations over the body, a distressed full feeling in the head, precordial oppression, or a constricted feeling in the chest, or dyspnea, and (highly characteristic) an acute severe pain in the lumbar region. Vasomotor disturbances quickly follow, especially a marked flushing of the face, or of the body generally. Collapse often follows, with a rapid feeble pulse, of low tension, a cold clammy skin, often nausea and vomiting, and sometimes syncope. Later, but within an hour, there is a violent chill, with a sharp rise in temperature, nausea and vomiting, and sometimes delirium. The first specimen of urine voided usually contains hemoglobin. Jaundice often develops after 24 hours, due in part to the rapid blood destruction, and in part to injury to the liver, which shows extensive focal necroses at autopsy. Urticaria is common at any stage of the reaction, but may occur in the absence of any other symptoms, and is not necessarily of significance.

The outlook in general is fairly favorable. A majority of the cases recover, even after stormy symptoms. Death may occur from circulatory collapse early in the reaction, or it may follow late from

injury to the liver and kidneys. In general the larger the amount of blood injected, the worse the outlook. At the first suggestion of such a reaction the transfusion should be stopped and epinephrin administered in dose of 1.0 cc. of 1 to 1,000 dilution by intramuscular injection, or in grave emergencies 0.2 to 0.3 cc. intravenously. The dose should be repeated at intervals of about ten minutes until the alarming symptoms of collapse have subsided. Epinephrin is of the greatest service in combating this phase of the reaction, but is of no avail in preventing injury to the liver, etc. Injury to the kidney may be lessened by the liberal administration of fluids and alkali.

There is great variation in the acuteness and severity of the symptoms following the injection of incompatible blood. In many cases the symptoms are relatively mild, and they may be insignificant. This doubtless depends on the rate at which the injected cells are destroyed. It is probably true that in general the most acute reactions are apt to occur in patients whose blood shows strong hemolytic and agglutinating activity. However, severe reactions certainly occur in the absence of hemolysins demonstrable in vitro and there is no close parallelism with the agglutination-titer. While the occurrence of agglutination has been emphasized as of crucial importance in tests for compatibility, it by no means follows that the symptoms of a reaction to incompatible blood are necessarily caused directly by intravascular agglutination of the injected cells. It is more probable that they are due to certain poisonous products of hemolysis. Even

in those cases showing no notable immediate reaction the incompatible red cells injected are destroyed in the course of a few days and are not of the slightest benefit to the patient. A second injection of incompatible blood is likely to be followed by a more violent reaction.

In some patients reactions occur with symptoms that suggest hypersensitiveness to a foreign protein, but without hemolysis or hemoglobinuria. There may be marked vasomotor disturbances with flushing or urticaria, or there may be severe circulatory depression with tachycardia and a fall in blood pressure. In rare instances death has occurred. It is thought that these may be reactions of an anaphylactic type to substances dissolved in the plasma. The blood of certain donors seems especially prone to cause such reactions, but hypersensitive patients often react in this way to blood from any source. No means of anticipating and avoiding these reactions is known.

When repeated transfusions are given a certain number of patients tend to react with increasing severity to each successive transfusion. This is particularly true in the case of pernicious anemia, purpura hemorrhagica, and leucemia. At first these reactions resemble severe reactions of the simple febrile type, but if transfusions are continued more severe or fatal reactions may occur, accompanied by hemoglobinuria and jaundice, even though the donor belongs to the same blood group as the recipient, and no sign of incompatibility can be made out in the preliminary tests. The writer has seen several

such cases and recently had an opportunity to study one such carefully. Reëxamination of the blood of both patient and donor, taken both before and after the transfusion, failed to show any trace of cross-agglutination or hemolysis. (Both belonged to Group O.) Injections of serum and whole blood of the donor and other Group O individuals failed to show any cutaneous hypersensitiveness on the part of the recipient. There is at present no way to anticipate these reactions. They rarely occur until from 10 to 20 transfusions have been given. Some patients, on the other hand, can tolerate apparently an unlimited number of transfusions, 50 to 100 or more, without noteworthy reactions.

INDICATIONS FOR TRANSFUSION

Transfusions in the main serve only to supply a deficiency in one or more of the constituents of the blood. They may be indicated to restore a diminished blood volume after hemorrhage, to replenish an inadequate store of red cells in anemia, or to introduce blood platelets and possibly other coagulation factors in the hemorrhagic diseases. It is useless to attempt to introduce leucocytes by transfusion. More than this transfusions cannot do, except that at times they probably exert a direct stimulating effect on the bone marrow. It is generally believed that so far as this stimulation is concerned small transfusions are as effective as large ones. On the other hand, if a plethora is created by excessive transfusion the activity of the marrow is distinctly inhibited.

Transfusions have been tried extensively in post-operative shock, but the consensus of opinion seems to be that they are of little value unless shock is associated with hemorrhage. Transfusions will not save or prolong the life of a dying patient unless anemia or hemorrhage is a significant factor in causing death. They are of no value in septic infections, either acute or chronic, unless there is an anemia or a tendency to bleed. If anemia is present they may be distinctly indicated, although the relatively frequent occurrence of severe febrile reactions in cases of sepsis must be considered. The results obtained by the transfusion of blood from immunized donors in general have been no better than with normal blood.

Spectacular benefit is obtained from transfusion in the collapse following acute hemorrhage, and in hemorrhagic disease of the new-born. Temporary cessation of bleeding may be expected in most cases of thrombopenic purpura and hemophilia. Great symptomatic relief may be obtained quickly in many chronic forms of severe anemia. Even in the presence of incurable disease they may be desirable in order to prolong the comfortable portion of a patient's life. In cases of chronic anemia of a relatively mild degree one or two transfusions may initiate an improvement which may be continued and maintained by other measures. Transfusion may make possible and safe a needed surgical operation which otherwise would be quite out of the question, either because of anemia or bleeding. In other conditions the benefits of transfusion are less

definite. In many cases the desirability of transfusion will depend on the skill and experience of the operator, especially on his ability to select compatible donors.[4]

[4] For a general summary and bibliography see: Beck, A., "Die Methodik der Bluttransfusion und die Vermeidung ihrer Gefahren," *Ergeb. d. Inn. Med. u. Kinderheilk.*, 1926, xxx, 150.

CHAPTER XXII

THE TECHNIQUE OF BLOOD EXAMINATIONS

EXAMINATION OF A FRESH DROP

FOR the purpose of making quickly a general survey of the formed elements of the blood, the examination of a fresh drop of blood is most valuable. It yields a great deal of information with a minimum expenditure of time and effort. If it be made a part of the routine examination of every patient many superfluous blood counts can be avoided, and but few cases needing a more thorough study will be overlooked.

A freely flowing drop of blood about 2 mm. in diameter is touched with the middle of a clean thin coverslip, care being taken to avoid touching the skin. The coverslip is immediately inverted on a clean glass slide, and the drop is allowed to spread under the weight of the coverslip. No pressure is permissible as it will crush or distort the cells. Air bubble formation can be avoided by tilting the coverslip slightly as it is lowered onto the slide. Glass ware must be perfectly clean and free from grease and dust. The oil immersion lens should be used, but the objective must not be allowed to strike the coverglass, and the oil must not run over the edge of the coverglass.

In a satisfactory preparation the red cells in the

central portion of the drop should be evenly distributed in a single layer, none on edge, and not crushed or distorted. In such a preparation one can determine satisfactorily the shape, size, and depth of color of the red cells. Any notable degree of anemia is at once apparent. The characteristic changes of the cells in sickle cell anemia can be made out satisfactorily only in such preparations. From the relative number of red cells and white cells one can determine the existence of any significant leucopenia, or leucocytosis. One can recognize the granular leucocytes (neutrophiles and eosinophiles) and differentiate them from the lymphocytes and monocytes. The differentiation of monocytes from large lymphocytes is more difficult. Fresh preparations are also valuable for the study of nucleated red cells and malarial parasites, but the detection of these objects is much easier in stained preparations.

If a fresh preparation is examined on a warmed stage (37° C.), the various forms of motility of the different types of leucocytes can be observed. Much new and valuable information can be obtained by a study of fresh preparations supravitally stained with neutral red or janus green by Pappenheim's method, as elaborated by Sabin and her associates. Examination of fresh preparations with dark field illumination opens up another entirely different field of study, and gives one a conception of the structure of these cells unattainable by any other method. However, a detailed discussion of these methods is outside the scope of this work.

The beginner is warned not to mistake for nuclei

or parasites the degenerative changes that take place in the substance of the red cells after fresh preparations have stood for a little time. The more common forms are:

(1) Crenations. The corpuscles shrivel and assume a thorn apple appearance. In the initial stage of the process one or two crenations projecting upward from the flat surface of the cell may have a deceptive appearance.

(2) Endoglobular degenerations of Maragliano. These are relatively large, roughly spherical, clear, highly refractile bodies that look like vacuoles. They vary in size from about one-fourth to three-fourths the diameter of the red cell. They tend to be centrally located, but the smaller ones may change their position in the cell. Unlike parasites they increase in size and number as the preparation stands. They can definitely be recognized by the fact that it is impossible to bring them sharply into focus. Change of focus results only in an apparent change of size, and does not make their outline clear.

(3) "Bacillary" degenerations. These are small, clear, refractile structures, usually rod-shaped, and having roughly the outline of a coarse bacillus. Globular and crescentic forms also occur. They may change their position in the cell so rapidly as to simulate active motility. Some look like slits in the cytoplasm of the cell.

(4) The hemoglobin may become concentrated in the center of the cell, making this region look dark and swollen, while the periphery looks pale and thin.

None of these structures have any pathological or diagnostic significance. Several times they have been described erroneously as parasites.

ENUMERATION OF THE RED BLOOD CELLS

The apparatus consists of a pipette for the accurate dilution of the blood, a special glass slide, or counting chamber, and a heavy perfectly plane coverglass to cover the chamber. The best type of chamber is the Hausser type of the Bürker chamber, with open moat and two ruled platforms. The improved Neubauer ruling is desirable. The glass slide, which is quite small, is carried by a bakelite holder which substantially lessens the risk of breakage. The chamber is cut from a single piece of glass. The ruled platforms are elongated rectangles in shape, their long axis is in the longitudinal axis of the slide, and they extend to the end of the slide proper. They are separated from each other by a deep transverse moat, and on either side they are separated by a pair of longitudinal moats from the four small supporting surfaces, which are so ground that they hold the bottom surface of the coverslip exactly 0.1 mm. above the ruled areas.

The coverslip and slide must be clean and free from dust and lint. First, the coverslip, which is a little shorter than the chamber, is placed on the slide, and is conveniently held in place by small metal clips provided for the purpose. The use of the clips is not essential. As the supporting surfaces have a dull ground-glass finish, Newton's rings will not appear. The pipette (Thoma type) is filled by gentle suction to the 0.5 mark with blood from a

freely flowing drop. If the blood is drawn a shade too far into the pipette it may be brought back to the mark by drawing the finger across the tip of the pipette while held tip downward, in a vertical position. The use of a Trenner automatic pipette avoids this difficulty. With the pipette held horizontally, the blood is wiped off the outside of the pipette, and the tip is plunged into the diluting fluid, in a small bottle with a wide mouth, and fluid is sucked up until the pipette is filled to the 101 mark.[1] During this process the pipette should be held vertically, and gently twirled between the fingers. If the glass bead tends to stick to the sides of the bulb, or to float on the surface of the fluid, it should be dislodged by tapping the pipette with the finger. When filled the pipette should be changed to a horizontal position, removed from the diluting fluid, the tip covered with the finger, and the pipette should be shaken for a few seconds to insure immediate mixing of the blood. The procedure up to this point must be carried out rapidly, otherwise the blood may begin to clot in the stem of the pipette and an even mixture of the red cells will be impossible. Time-consuming efforts to secure great accuracy in the dilution are likely to introduce gross errors for this reason. The pipette may now be laid aside for a few hours if it is not convenient to complete the count at once.

The pipette is next held horizontally with the

[1]As diluting fluid Hayem's solution is recommended: Sodium chloride 1.0 gram, Sodium sulphate 5.0 grams, Mercuric chloride 0.5 gram, Distilled water 200 cc. This must be filtered occasionally to remove a precipitate which tends to accumulate and makes counting difficult.

tip closed by the finger, and shaken vigorously in a direction at right angles to its long axis for two or three minutes. Several drops are then blown out and discarded, the tip of the pipette is touched to the surface of the ruled platform of the counting chamber at the edge of the coverslip, and a little of the diluted blood is allowed to flow out, or is gently blown out, so that it is carried by capillary attraction under the coverslip. The quantity should be sufficient completely to cover the surface of the ruled platform but should not overflow into the moats. Should it flood to such an extent that it covers the other platform, or gets between the coverglass and the supporting surfaces, the preparation is ruined, the counting chamber must be cleaned and the process repeated. Some little practice is necessary to fill the chamber satisfactorily. If the fluid flows out too rapidly flooding is likely to occur. If it flows out too slowly air bubbles are likely to be caught under the coverslip and the preparation ruined in this way. If the first platform is covered satisfactorily, the second one is similarly covered. There must be no delay between shaking the pipette and filling the chamber. Should there be any interruption the pipette must be reshaken.

The slide is then allowed to stand for two or three minutes, so that the red cells may settle out. It must be placed on some perfectly horizontal surface, preferably the microscope stage. Should it become necessary to let the preparation stand for more than a few minutes it must be put in some kind of a moist chamber to prevent evaporation. The preparation is then inspected under the low

power to make sure that the cells are evenly distributed over the ruled areas, and that there are no air bubbles. An uneven distribution is probably due to grease on the counting chamber or cover-glass. Both should be thoroughly cleaned, the pipette again shaken, and the process repeated. If the preparation is satisfactory one then proceeds with the count, using the high power dry lens. The counting is greatly facilitated by using a mechanical counter. A mechanical stage is convenient but not essential.

In making a red cell count only the central square millimeter of the 9 sq. mm. of the ruled area is used. This area is subdivided into 400 small squares. In the improved Neubauer ruling these 400 small squares are arranged in 25 groups of 16 small squares each by cutting an additional line very close to each fifth vertical and horizontal line, thus making a double line. One counts the red cells in 5 of these groups of 16 small squares each, preferably the four corner groups and the central group, to insure as representative a distribution of the cells as possible. The second ruled area is similarly counted, and if the two figures correspond well, the average is taken. The two figures should not differ by more than 30 cells. In making the count any white cells which may be present are counted in as red cells. Should the white cell count be very high, this may be subtracted later from the total count, but this is rarely necessary. Cells touching either the left-hand line, or the upper line bounding a square are counted as within the square, and cells touching the right-hand line, or the lower line

are not counted, regardless of where the bulk of the cell may lie.

Calculation: The area of one of the smallest squares is 1/400 of a sq. mm. Five groups of 16 such squares in each have been counted; that is 80/400, or 1/5 of a sq. mm. The thickness of the layer of diluted blood on the ruled platform was 0.1 mm. The dilution of the blood in the pipette was 0.5 in 100, or 1 in 200. The number of cells counted (the average of the two fields), therefore, is multiplied by 5, by 10, by 200; that is, by 10,000. Hence the total count may be obtained simply by adding four ciphers to the average count of the two fields.

The probable error in the red cell count depends entirely on the skill and care used in making it. With a fair degree of experience and reasonable care the probable error is about 4 per cent, or 200,000 cells in a total of 5 millions. With much experience and painstaking care this may be reduced to within 2 per cent. The average routine count, rather hurriedly and carelessly made, may easily show an error of 5 to 10 per cent.

ESTIMATION OF HEMOGLOBIN

The advantages of the Sahli hemometer are: (1) It is reasonably cheap, and not very fragile. (2) It is simple to manipulate, and the readings can be made in a few minutes. (3) The color standard is a tube of acid hematin suspension, identical with the substance (blood) to be tested, and therefore fairly accurate matching can be made in any reasonably good light. The disadvantages are: (1)

The standard color tube fades with age, quite rapidly if exposed to light. (2) If either the standard tube or the dilution tube is broken, and a new one substituted, the instrument will no longer give accurate readings unless restandardized, because of the practical impossibility of finding tubes of exactly the same diameter. (3) The result is expressed in terms of percentage of normal (instead of grams per 100 cc.), and the normal standard adopted is too high. It is, therefore, necessary to use a correction factor for a new instrument. Frequent restandardization is also necessary for even approximate accuracy, and if this is neglected errors of 20 per cent or more may develop in the readings.

The instrument consists of a sealed glass tube containing an acid hematin suspension corresponding to a 1 per cent dilution of normal blood; a graduated dilution tube of the same internal diameter; a black bakelite stand with a ground-glass plate as back, in which the colors may be compared; and a small pipette for the accurate measurement of 20 c. mm. of blood.

In making a test the sealed glass tube, containing a glass bead, is removed from the stand and gently inverted several times, to insure even distribution of the acid hematin, which is in fine suspension, not in solution. Shaking must be avoided or air bubbles will be formed, which seriously interfere with the reading. The graduated tube is filled to the 10 graduation with N/10 HCl. Great accuracy in the strength of the acid solution is not essential. The ear, or preferably the finger tip, after being cleaned

with an alcohol sponge, and dried, is given a fairly deep prick with a blood lancet. The finger must be warm and pink, not cold and white or blue. If cold it should be held in warm water until active circulation is established. The blood must flow freely, preferably without any pressure. Occasionally a slight degree of pressure is necessary to secure a flow, but anything more than the gentlest pressure results in stasis, with a tendency to make the readings for hemoglobin and red cell count too high. Another deeper puncture should be made if necessary. The first drop is discarded. Blood is then drawn accurately to the mark (20 c.mm.) in the small pipette, using the finger tip if necessary to bring the blood exactly to the mark. Great care must be taken that all blood adhering to the outside of the pipette is wiped off. The blood in the pipette is then gently blown into the acid solution in the graduated tube, thoroughly mixed, and the pipette is rinsed out several times with the solution.

The acid changes the hemoglobin of the blood into acid hematin, with the development of a brown color. The development of this color is a gradual process. The rate of change is rapid at first, and becomes progressively slower as the maximum development of color is approached. The maximum color is not reached until after standing 24 hours. The result obtained may, therefore, vary greatly with variation in the time the mixture stands before the reading is made. The instructions given with instruments are to make the readings exactly 60 seconds after the blood is added to the acid. This is quite satisfactory provided this time interval is

always rigidly adhered to. In actual practice it is rarely practicable to do this, and the writer strongly recommends that the mixture be allowed to stand fully ten minutes, when about 95 per cent of the maximum color will have developed. The maximum color can be obtained by placing the tube in a water bath at about 56° C. for 7 minutes or more, and this is desirable for the most accurate results. Whichever time interval is chosen, it must be adhered to consistently, and must be observed in the preliminary standardization of the instrument.

After the expiration of the chosen interval, or after the maximum color development has been secured, the blood mixture is diluted drop by drop with distilled water until the color matches that in the standard tube. The contents of the tube must be well mixed after each addition of diluent, preferably with a fine glass rod. The pipette is not satisfactory for the purpose, and if the tube is inverted against the finger some of the fluid will be lost. As in any colorimetric determination care must be taken not to fatigue the eye by too prolonged observation of the tubes. After 10 or 15 seconds' observation the eye should be rested for an equal interval.

After the reading is finished the pipette should be cleaned and dried with water, alcohol, and ether, the tube rinsed out with water, and the instrument put away out of the light, to avoid rapid fading of the standard tube. The dilution tube should occasionally be wiped out with a cotton swab, or cleaned with acid cleaning solution to remove the

precipitate of acid hematin which gradually accumulates in the tube with use.

The reading obtained must be multiplied by the correction factor for the instrument, which is determined by standardizing the instrument by one of the methods to be described. The ideal method of standardization is to make several readings of a normal blood with the instrument to be tested, and compare the average of these readings with the hemoglobin content of the same blood as determined by Van Slyke's method. If this is not practicable, these readings may be compared with readings of the same blood made with an instrument which has been shown to be accurate by the Van Slyke method. If the blood gives a reading of 100 per cent (15.6 grams per 100 cc. of blood) on the instrument of known accuracy, and a reading of 90 per cent on the instrument to be tested, the standard tube of the latter instrument is evidently too dark, and readings with it will be too low unless corrected. The correction factor is found by dividing the reading on the correct instrument by that on the other; in this case, 100/90, or 1.11. All readings on the instrument being tested must be multiplied by 1.11 to obtain the correct figure. Or if the blood gives a reading of 95 per cent on the correct instrument and of 110 per cent on the instrument to be standardized, the standard tube of the latter is evidently too pale, and readings with it are too high, as is apt to be the case with instruments that have been in use. The correction factor is determined in the same way, by dividing the reading on the correct instrument by that on the one to be corrected; 95/110,

or 0.86, is the correction factor, and all readings must be multiplied by 0.86 to get a correct result.

If an instrument of proven accuracy is not available, anyone who can make a hemoglobin determination at all can make a fairly satisfactory correction quite simply. Carefully determine with the instrument the hemoglobin content of the blood of several normal individuals whose red cell count has been shown to be 5 million cells, or close to this figure. Assume that the average reading of these bloods would be 100 per cent on a correct instrument. If the average on the instrument being tested is not 100 per cent, calculate the correction factor by dividing the (assumed) correct reading, 100 per cent, by the average of the readings actually obtained. Henceforth multiply all readings by this factor to get correct results. While this method falls short of research accuracy, it will prevent serious errors creeping into the determinations. Instruments which are much used, and exposed to light, should be restandardized at least once in three months.

Recently a new form of the Sahli instrument has been put on the market, in which the standard color tube has been replaced by a colored glass rod which, it is claimed, does not fade. However, this form of the instrument has some serious drawbacks. The color match, while good, is not as good as that of the old form of the instrument. Furthermore, as one is comparing the color intensity of two different substances (glass tube, hematin suspension), the apparent relative intensity of their color will vary considerably if the source and character of the light

is changed. Thus, if readings are made by daylight the writer gets results fully 10 per cent lower than when read by light from a Mazda lamp. It is, therefore, necessary to use one source of light uniformly, or to determine a correction factor for each different source of light used. Preliminary standardization of a new instrument is just as important as with the old type. However, if occasional restandardization is a serious difficulty, this new type of the instrument will probably be preferable to the old one.

If the instrument has been properly standardized, the probable error in the estimation will be from 2 to 5 per cent, depending on the care and experience of the observer. If standardization is neglected, errors of 10 to 20 per cent are not infrequent.

The use of the Tallqvist hemoglobin scale of colored papers is not recommended. If the colors have not faded with age and exposure to light readings on approximately normal blood can be made with an error of less than 10 per cent. For blood with less than 60 per cent of hemoglobin the results are little better than a guess.

DETERMINATION OF THE VOLUME OF THE RED BLOOD CELLS

For this, one requires a fairly powerful centrifuge capable of a speed of at least 2,500 revolutions per minute, and special accurately graduated centrifuge tubes. For ordinary purposes the Van Allen hematocrit tube is recommended. This tube resembles in its construction a large blunt-tipped hemacytometer diluting pipette. Blood from a large freely

flowing drop is drawn by suction to the upper (100) mark on the stem of the tube. Isotonic (1.3 per cent) sodium oxalate solution is then drawn up through the graduated stem until the bulb above is about half full. The exact amount of the diluent it not important. The tube is then closed by stretching a stout rubber band over both open ends, and it is centrifugalized at 2,500 or more revolutions per minute until the volume of red cells which settle into the lower part of the graduated stem has reached a constant volume. The height of this column is then read, and expresses directly the percentage by volume of the blood which is made up by its red cells. This figure is spoken of as the volume percentage of red cells.

RETICULATED RED BLOOD CELLS

These require special "vital" staining methods for their demonstration. The following simple method is usually satisfactory. Two solutions are required: (1) 1 per cent solution of brilliant cresyl blue in 0.85 per cent salt solution. The cresyl blue is ground in a mortar with a few drops of salt solution, the balance of the solution gradually added, and the final solution filtered. This deteriorates gradually, but can generally be used for several weeks. (2) 1 per cent solution of potassium oxalate in 0.85 per cent salt solution. Just before use one part of stain is added to 5 parts of oxalate solution. To 25 drops of diluted stain is added one drop of blood (several drops if the red cell count is low), the contents of the tube are mixed, and allowed to stand at least 20 minutes. Even better results are

obtained if the tube is left in the ice box for from 1 to 18 hours. The tube is centrifugalized just long enough to throw the cells down but not to pack them; a few seconds suffice with a good centrifuge. The supernatant fluid is poured off, the sediment is suspended by agitation in the drop or two of fluid which adheres to the walls of the tube, and films of this sediment are made in the usual way. They are dried in the air, and without further staining are mounted in balsam and examined with the oil lens. The number of reticulated cells per 1,000 erythrocytes is determined by an actual count. In order to reduce the size of the microscopic field to practicable dimensions for a count, a piece of stiff paper with a square hole ¼ inch in diameter cut in the center should be trimmed to fit and inserted in the eye piece.

Instead of using solutions, coverslips (cleaned) may be painted with a thin layer of 0.5 per cent alcoholic solution of brilliant cresyl blue, and dried. Blood films are made in the usual way, using these stain-coated coverslips. The air dried films may be mounted in balsam and examined, or they may be counterstained with Wilson's stain. In general it is easier to demonstrate these cells in cases of anemia, in which they are increased in number, than in normal blood.

ENUMERATION OF THE WHITE BLOOD CELLS

The apparatus required is the Thoma diluting pipette for white corpuscles, and the same counting chamber used for counting red cells. The pipette is filled to the 0.5 mark in the same way, and dilut-

ing fluid [2] is drawn up to the 11 mark. The dilution is therefore 1 in 20. The pipette is shaken and the two platforms of the counting chamber are covered with the diluted blood in the same manner as in making the red cell count. The count is made with the low power objective. The entire ruled area of 9 sq. mm. is inspected, and if the distribution of cells is even throughout this area the cells are counted in 5 of the 9 sq. mm. areas on each of the two platforms. To get the most representative distribution of cells it is preferable to count the four corner squares and the central square. Calculation: The sum of the counts of the two fields represents the number of cells in 1 c.mm. of diluted blood, since the total area counted was 10 sq. mm., and the thickness of the layer was 0.1 mm. The actual leucocyte count per 1 c.mm. of blood is obtained by multiplying this sum by the dilution of the blood, which is usually 20. Nucleated red cells, if present, are counted in as leucocytes. In rare cases in which large numbers of nucleated red cells are present a correction may be necessary. The probable error in the count when made with reasonable care is about 5 per cent.

DIFFERENTIAL COUNT OF THE LEUCOCYTES

The purpose of a differential count is to determine the relative number of the different types of leucocytes present. For accurate differential counts well-made films on coverslips are essential. When

[2] Tuerk's solution is recommended: Glacial acetic acid 1.0, Aqueous gentian violet solution 1% 1.0, Water 100.0. This requires frequent filtration, as yeasts grow in the solution.

drawn or pulled on slides the larger cells tend to accumulate at the margins and especially at the end of the film, while the smaller cells (lymphocytes) are more uniformly distributed over the central parts of the film. The distribution of the cells, therefore, is seriously disturbed. Slides are satisfactory for a study of individual cells, and for a search for parasites.

Preparation of films. The first essential is clean coverslips. They should be ⅞ inch square, No. 1, of the best glass obtainable. A convenient and satisfactory method of cleaning is to use potassium bichromate-sulphuric acid cleaning mixture. The slips should be dropped one by one into the solution and allowed to remain for a day or longer. They are then removed, rinsed individually in running water, and dried from alcohol or hot distilled water, best with an old linen handkerchief. Before drying them the hands should be scrubbed to remove grease as completely as possible. If soap is used on the slips it must be rinsed off thoroughly with hot water. The use of jewelers' rouge to polish the surface of the slips is permissible but unnecessary. Throughout the drying process, and whenever the coverslips are to be handled later, coverslip forceps should be used and never the fingers, as the slightest trace of grease ruins the film. This is the chief stumbling block for the beginner in the way of making good films. The clean dry slips should be kept in some dust proof container.

When needed coverslips are removed from the box with forceps, one slip is grasped by one mar-

gin with a crossbill forceps, or by its edges between the thumb and index finger of the left hand, and any dust or lint that may be on it is carefully blown off or brushed off with a small camel's-hair brush that should be washed occasionally in ether to keep it free from grease. A second coverslip is taken up with forceps, or by its edges with the fingers of the right hand, brushed, and touched to a freshly exuded drop of blood about 2 mm. in diameter, not larger. This is quickly lowered onto the other coverslip, tilting it slightly in the process. The drop should spread evenly under the weight of the coverslip. No pressure is permissible. When the spreading of the drop begins to slow down, but before it has ceased, the upper slip is grasped again and is quickly pulled away from the lower slip in a direction parallel to their plane surfaces. Great care must be taken that the plane surfaces of the two coverslips be kept perfectly parallel with each other during the pulling, as any tilting or elevation of the upper slip from the lower one will ruin the preparation.

The chief causes of failure are:

Grease or dust on the coverslips.

Beginning clotting in the drop, either because the blood is not flowing freely, or because of slowness in the manipulations.

Too small, or, more often, too large, a drop is taken.

The slips are pulled apart too soon, giving too thick a film; or too late, causing crushing and distortion of the cells.

In pulling the coverslips apart one is tilted away from the other, giving an uneven distribution of the cells.

In a satisfactory film a considerable area in the center, preferably at least 8 low power fields, will show the red cells evenly distributed in a single layer, fairly close or even touching, but not heaped up or overlying each other, and the white cells will also be fairly evenly distributed, and but few crushed or distorted. The films are allowed to dry in the air, and must be protected from dust, and flies.

The most satisfactory stain for ordinary work is Wilson's stain or Wright's stain.[3] The coverslip is grasped by one corner in staining forceps of a type which will hold the slip in a perfectly horizontal position, and is covered with the stain (6 or 8 drops). After exactly one minute an equal volume of neutral distilled water, or preferably a buffered phosphate solution,[4] is added, taking care not to let the fluid run over the margins, and it is allowed to stand 5 minutes. By this time an iridescent scum should have formed on the surface of the fluid. The stain is then washed off by holding the slip in a perfectly horizontal position over a waste jar, and flooding it with distilled water or buffered solution. If the slip is tilted so as to al-

[3] The writer recommends the purchase of solutions of one of these stains, ready for use, from any reliable dealer in biological reagents.

[4] To one liter of water is added 6.63 grams of C.P. recrystallized acid potassium phosphate and 2.56 grams of anhydrous basic sodium phosphate. The solution should have a pH of 6.4 (Haden).

low the diluted stain to run off, the scum on the surface of the fluid will adhere to the film and ruin the preparation. The slip is then either blotted carefully or dried in air and examined. In a satisfactory preparation the red cells will be stained a pinkish-buff color, the nuclei of the white cells a deep purplish-blue, the granules of the neutrophilic leucocytes a pink, lilac, or violet pink, the platelets will be deeply stained, and there will be no precipitate on the film. If the nuclei are stained faintly or not at all, the difficulty is due to acid in the stain (or water). The buffered phosphate solution should be substituted for water in the dilution of the stain and washing the film. If this does not give satisfactory results a different preparation of stain must be secured. If the film is stained too blue it may be due to an alkaline preparation, or it may simply be overstained. In this case relatively less stain and more diluent may be used, the time of staining may be shortened and the time of washing lengthened. One may also decolorize rapidly with a few drops of 95 per cent alcohol, washing again immediately with water. If this be attempted the alcohol should not be in contact with the stained film for more than a second. The optimum degree of dilution of the stain and time of staining varies with different preparations of stain, and to some extent with different specimens of blood, and may have to be altered from the average figures given.

Best results are obtained with freshly made films. Films over 24 hours old yield much less satisfactory

preparations. Great care must be taken that no trace of water gets into the bottle of stain. Also it must not be exposed to acid fumes. Any trace of either ruins the stain. If satisfactory, the slip is mounted film side down in cedar oil, or if it is desired to keep the specimen for some time, in *neutral* balsam.

In making differential counts, as far as possible films should be selected which contain few smudges. The count should be made from those portions of the film which are of suitable thinness, so that the white cells are well spread out, but not crushed or distorted. To insure reasonable accuracy at least 250 cells should be counted. This usually suffices, but if the blood is abnormal a second count of 250 or more cells should be made, preferably from a second film, and the results averaged. Every cell encountered must be included. They should be classified under different subheadings for each of the 5 types of leucocytes met with in normal blood, for each immature or pathological form that may be encountered, and an additional heading for unidentified cells. Smudged cells should be included in their proper group when their identity can definitely be recognized, otherwise they should be classed as "unidentified" or "smudged." If they are merely omitted from consideration the percentage figures for the less frequent types of cells (eosinophiles, *e.g.*) will be too high. Nucleated red cells, if present, should be noted, but not included in the 250 cells on which the differential count is based. A total leucocyte count should always be

made when films are taken for a differential count, so that the absolute number of each type of cell per c.mm. can be calculated, and this number should be recorded, as well as the percentage of the total count made up by each type.

PEROXIDASE STAIN

The freshly made film is covered with Goodpasture's stain for one minute. This is then diluted with equal parts of water to which a little hydrogen peroxide has been added (about 2 to 5 drops to 5 cc. water) and stained for 5 minutes. The film is then washed with water, dried, and mounted. Different specimens of stain may require some change in the degree of dilution, the time of staining, or the amount of hydrogen peroxide used.

ENUMERATION OF THE BLOOD PLATELETS

For an actual count one takes a diluting pipette for red cells, moistens the interior with diluting fluid, and draws blood from a freely flowing drop up to the 1.0 mark. The bulb is then filled with diluting fluid in the usual way, the pipette at once shaken for about 3 minutes, and the counting chamber filled as for a red cell count. The chamber must stand for 15 to 20 minutes to allow adequate sedimentation of the platelets, and during this time must be put in some kind of a moist chamber to prevent evaporation. The platelets appear (under the high dry lens) as small highly refractile bodies from 1 to 4 microns in diameter. With normal blood the count offers little difficulty, but in patho-

logical blood with a reduced count and many minute platelets the count may be quite difficult. One must not confuse extruded leucocyte granules for minute platelets. In making the enumeration one counts the platelets in the same areas as in making a red cell count, and the calculation is the same, except that the dilution is only 1 to 100, instead of 1 to 200.

As diluting fluid 3.5 per cent sodium citrate solution is fairly satisfactory. The following solution is better, but must be freshly prepared daily.

Sodium citrate	1.5
Glucose	1.0
Sodium chloride	.85
Water	100.0

Brilliant cresyl blue sufficient to give the solution a bright blue color may be added to advantage.

VAN DEN BERGH REACTION FOR BILIRUBIN

(a) *Direct reaction.* To 1 cc. of clear serum (which must not be blood tinged) one adds 1 cc. of Ehrlich's diazo reagent. If there is an immediate appearance of a bright purplish red color which is maximal or practically so within a minute, one has a positive immediate direct reaction. Such a reaction is obtained only in case of obstructive jaundice. If the color appears only after standing for some minutes, one has a positive delayed direct reaction. Such a reaction has the same significance as a positive indirect reaction. Any serum which gives a

positive direct reaction will also give a positive indirect reaction.

(b) *Indirect reaction.* To 1 cc. of clear serum add 2 cc. of 96 per cent alcohol, and remove the precipitated protein by centrifugalization. To 1 cc. of the clear supernatant fluid add 0.5 cc. of alcohol and 0.25 cc. of diazo reagent. The (immediate) appearance of a reddish violet color indicates a positive indirect reaction. A serum giving only an indirect reaction is obtained in cases of jaundice due either to abnormal blood destruction, or to liver injury.

It is believed that in serum giving an indirect reaction only, the bilirubin is combined with serum proteins in such a way as to prevent its reacting with the diazo reagent until it is liberated by precipitation of the proteins with alcohol. In obstructive jaundice the bilirubin is free to react immediately with the diazo reagent. The change in the state in which the bilirubin exists is apparently brought about during the process of absorption and excretion by the liver. This reaction appears to be highly specific for bilirubin, and it seems to be dependable for the differentiation of obstructive jaundice from "hematogenous" jaundice.

The bilirubin content of the serum may be estimated by comparing the intensity of the color obtained in the indirect reaction with that of a 2.16 per cent solution of anhydrous cobalt sulphate (or a 3.92 per cent solution of the crystalline salt). It is assumed that the concentration of bilirubin after the addition of the various reagents is 1/5 of

that in the original serum. If the color exactly corresponds to that of the standard solution the serum is said to contain 5 units of bilirubin. One unit corresponds to a concentration of 1 in 200,000, or 0.5 mg. per 100 cc. Normal serum contains from 0.2 to 0.5 units.

ICTERUS INDEX

The Van den Bergh reaction is not a sensitive reaction, and is not entirely satisfactory for measuring relatively small amounts of bilirubin. For this purpose one should determine the icterus index of the serum (Meulengracht). The color of a specimen of clear serum, which must be free from any trace of dissolved hemoglobin, is compared with that of a 0.01 per cent solution of potassium bichromate (index 1). A colorimeter is desirable but a satisfactory estimation may be made simply by diluting 1 cc. of serum with 0.85 per cent salt solution in a small graduate or Wassermann tube until it matches the color of the standard solution in a similar tube of the same diameter. The number of times the serum is diluted expresses the icterus index. The only pigment that is likely to interfere is carrotin, hence the diet must have been free from carrots.

Normal serum has an icterus index of 4 to 6. If the index is above 15 (or about 2 units by the Van den Bergh test), clinical signs of jaundice appear. The range between 6 and 15 indicates a "latent jaundice," and these tests are of special value in detecting such slighter degrees of hyperbilirubinemia, which are often of great diagnostic importance.

BLEEDING TIME (*Duke*)

A moderately deep small cut is made in the skin of the ear or finger tip with a small scalpel or blood lancet. At intervals of half a minute the drop of blood exuding is removed by touching it with a piece of filter paper. The skin should not be touched or rubbed. This is repeated until bleeding ceases. In performing the test, incisions should be made at two or three different places. A single test may be deceptive. In normal individuals the bleeding time is relatively independent of the size and depth of the cut. In some pathological cases this is not true, and annoying bleeding may occur from an unnecessarily large cut. The normal bleeding time is from 1 to 3 minutes. A bleeding time of 10 minutes is moderately but definitely prolonged. In marked cases bleeding may continue for an hour or more. A prolonged bleeding time is characteristic of thrombopenic purpura.

COAGULATION TIME (*Howell, modified by Lee and White*)

A 10 cc. Record syringe is sterilized, a sharp needle attached, and the syringe is rinsed out with sterile salt solution, the last of the solution being expelled with the syringe held vertically, point up, so that the needle and the dead space in the tip are filled with solution. With the usual aseptic precautions the needle is inserted very carefully into a vein, and 10 cc. of blood are slowly aspirated. A tourniquet may be used. The success of the test

depends entirely on entering the vein cleanly at the first attempt. If this is done clumsily so that tissue juice is mixed with the blood, the result is worthless (the coagulation time will be shortened). The needle is then removed and 1 cc. of blood is gently expelled into each of three or four "Wassermann" tubes, and also into one or two additional tubes to which 3 drops of 1 per cent calcium chloride solution have been added. These tubes should have an interior diameter of about 1 cm. and must be chemically clean (acid cleaned) and rinsed out with salt solution. If larger tubes are used the coagulation time will be longer.

The tubes should be left undisturbed in a rack for 5 minutes from the time the blood first entered the syringe. The first tube is then removed from the rack and tilted slightly to determine whether the blood is still fluid. After a half minute the second tube is similarly examined, and after a similar interval the first is again observed. This is continued until the tube can be inverted without spilling the blood. The third tube (hitherto undisturbed) is then examined. Quite frequently the blood in it will not have coagulated, and if so it is similarly observed until it also can be inverted. Errors in technique usually tend to make the coagulation time too short. Normally the coagulation time is from 6 to 12 minutes (more often 12 than 6). Slight prolongation is of little or no significance. The time is markedly prolonged in hemophilia, often over an hour. There is no parallelism between the bleeding time and the coagulation time.

If clotting occurs in the tubes to which calcium chloride solution has been added before it does in the others, there is a deficiency of available calcium in the blood. With normal blood the addition of this quantity of calcium chloride solution is apt to prolong the coagulation time somewhat. (Lee and Vincent's "calcium in vitro test.") [5]

PROTHROMBIN TIME (*Howell*)

Eight cc. of blood obtained as described above for testing the coagulation time are expelled at once into a centrifuge tube containing 1 cc. of a 1 per cent solution of potassium oxalate in 0.85 per cent salt solution. The tube is inverted once to mix, and it is then centrifugalized at high speed until the supernatant plasma is clear. A series of 8 cleaned tubes is prepared. The first is retained as a control. To the others are added 0.5 per cent calcium chloride solution in increasing quantities, from 2 drops to 8 drops, respectively. To each of the 8 tubes 5 drops of clear plasma are then added and the time noted at which coagulation has so far progressed that the tubes can be inverted. The minimum coagulation time with normal blood in this test is about 12 minutes, and it is observed in the tubes containing the smallest amount of calcium chloride solution (2 or 3 drops). This minimum time is greatly prolonged (an hour or more) in hemophilia. If there is a lack of available calcium, the minimum time (which may be normal) will be

[5] Lee, R. I., and White, P. D., "A clinical study of the coagulation time of the blood," *Am. Jour. Med. Sci.*, 1913, cxly, 495.

observed in tubes containing larger amounts of calcium chloride.

ESTIMATION OF FIBRINOGEN (*Whipple*) [6]

The fibrinogen is determined as fibrin. Nine cc. of blood obtained as in the preceding tests are mixed with 1 cc. of 1 per cent potassium oxalate solution and centrifugalized at high speed until the cells are completely sedimented. The relative cell volume and plasma volume are noted. Two cc. of the clear plasma are put in a beaker with 40 cc. of 0.85 per cent salt solution, and 2 cc. of 2.5 per cent solution of calcium chloride is added. (Plasma obtained for the determination of the prothrombin time may also be used.) After two hours, when the solution has clotted throughout, the fibrin is freed from fluid by gentle manipulation and pressure with a glass rod. The relatively small mass of fibrin is washed thoroughly in water. It may then be transferred to a crucible, dried at 110° C. in a drying oven (3 to 10 hours) to constant weight, and the quantity thus directly determined. Or the moist clot may be transferred to a large ignition tube, heated with 1 cc. of Folin's diluted acid digestion mixture, and the nitrogen content determined just as is the nonprotein nitrogen of a blood filtrate by the Folin-Wu method. The protein (fibrin) content of the 2 cc. of plasma is 6.25 times the observed nitrogen content. From this the fibrin, and substantially the fibrinogen, content of the whole

[6] Foster, D. P., and Whipple, G. H., "An Accurate Method for the Quantitative Analysis of Blood Fibrin in Small Amounts of Blood," *Am. Jour. Physiol.*, 1922, lviii, 365.

blood may readily be calculated. The normal figure is about 0.4 per cent (400 mg. per 100 cc.). Only marked variations are significant.

DETERMINATION OF THE BLOOD GROUP

One prepares a cell suspension of the patient and of each prospective donor by adding one or two drops of blood to 5 cc. of 1 per cent solution of sodium citrate in physiological salt solution. One also secures enough blood by any convenient method to yield several drops of clear serum. Sufficient blood can be obtained from an ear prick in a Wright tube. One drop of cell suspension is added to a drop of serum, either with a platinum loop or with a capillary pipette. This is mixed and a small drop of the mixture is placed in the center of a clean coverslip, which is mounted preferably on a hollow-ground glass slide, and sealed with oil. The preparation should be inspected shortly after being set up. There should be a sufficient number of red cells present to form a single layer of cells after they have settled down to the surface of the drop, with the cells close together or touching, but not heaped up.

The following preparations are set up with the cells of each of the individuals in question:

(1) Unknown cells + serum of Group A (2)
(2) Unknown cells + serum of Group B (3)
(3) Unknown cells + serum of Group O (4)

The third preparation is not essential, but is a valuable check on the accuracy of the observations

of the first two preparations. The activity of the standard sera used must be checked by occasional tests with agglutinable cells.

The Group is determined by the result of the first two preparations, and may be read quickly from the following outlines:

Serum A (2)	Serum B (3)	Group of cells tested
+	+	AB (1 Moss)
o	+	A (2)
+	o	B (3)
o	o	O (4)

In preparations in which no agglutination occurs the cells remain evenly distributed for hours. If agglutination occurs the cells become clumped together irregularly in compact masses. In most cases the agglutination occurs within a few minutes, and is quite unmistakable. It may be evident as soon as the preparation can be examined, and the clumps may be so large as to be visible to the naked eye. As a rule they are quite evident within 5 to 10 minutes. If the clumps are indefinite or small, the mixture should be agitated gently by tilting the slide slightly from side to side. If agglutination is to occur at all, the cells nearly always will then settle out conspicuously and compactly clumped. However, if still negative, the preparation should be incubated and examined after the lapse of at least one hour.

On the basis of these tests one or more of the prospective donors are selected who belong to the same group as the patient, or if this is not possible

a Group O donor is chosen and the following test is set up:

(4) Recipient's serum + Donor's cells.

If no agglutination or hemolysis occurs in this preparation, the donor may be used with confidence that the blood is compatible.

However, in a few Group O individuals the agglutinin titer of the serum is so high that agglutination is to be expected in the dilution which exists when a pint of blood is injected into an average patient (1 in 7 to 1 in 12). Such individuals have been characterized as "dangerous universal donors," on the assumption that agglutination in the body parallels agglutination in vitro, and that such an agglutination (of the recipient's cells by the donor's serum) will cause a serious reaction if it occurs. While the writer knows of no positive evidence of the actual occurrence of such reactions, and has often used Group O individuals as universal donors without regard to the agglutinin titer of their serum, the theoretical possibility of danger must be recognized. Therefore, when a Group O individual is to be used for the first time as donor for a patient of a heterologous group, for complete safety the agglutinin titer of his serum should be tested, and if in dilution of 1 in 10 or higher it causes agglutination of the recipient's cells, the donor should be rejected.

Some workers prefer to seal such mixtures in capillary tubes and let them stand overnight in the ice box. This is but rarely of real advantage if the method above outlined is carefully followed. It is

quite unnecessary if group determinations are being made with stock sera of which the agglutinating activity has been demonstrated, but it may be desirable in the study of puzzling cases.

It is possible also to determine the group by testing the (unknown) serum with known Group A and Group B cells, or cells and serum may be tested with serum and cells from a known Group A or Group B individual. The method recommended is preferable. It is easy to obtain serum from individuals known to belong to Group A and Group B, and to preserve it in active form for a long time, provided it is sterile and kept in the ice box. Cells can be preserved for a few days only. This method avoids possible errors which may result from feeble agglutinating activity in the serum to be tested. Although agglutination usually occurs promptly, and is a conspicuous and unmistakable phenomenon, it is not rare to find an individual who clearly belongs to Group O because of the insusceptibility of his cells to agglutination in any other human sera, but whose serum shows feeble or negligible agglutinating activity in vitro. Even if "cross-matching" is carried out with the cells of the donor, agglutination may be overlooked for the same reason. However, if such an individual is given a transfusion of incompatible blood from a Group AB, Group A, or Group B donor he will usually react to it violently. The writer has seen a number of reactions due to this particular error.[7]

[7] Clough, P. W., and Clough, M. C. "A study of the reactions following the transfusion of blood." *South. M. J.*, 1921, xiv, 104.

Agglutination may be missed when fresh serum is used, if it contains an active hemolysin so that a large number of the red cells are dissolved. If the preparation is watched, agglutination will usually be evident before hemolysis has occurred. If not, there will be a conspicuous diminution in the number of cells present, and the fluid may be visibly tinged with hemoglobin. This difficulty may be obviated by repeating the test using serum that has been heated at 56° C. for 20 minutes. This destroys the complement necessary for hemolysis, but does not interfere with agglutination.

On the other hand, agglutination may erroneously be assumed to be present. If fresh serum is used, rouleaux formation often occurs and may be mistaken for agglutination. This rarely confuses the experienced observer, since the appearance of the aggregations of red cells is usually quite different. In rouleaux the cells are assembled regularly, face to face, in longer or shorter columns, like stacks of coins, whereas in real agglutination the clumps are more compact, the arrangement of the cells in the clumps is absolutely irregular, and the shape of the cells is often more or less distorted. Stirring or agitating the mixture definitely makes agglutination more conspicuous, but tends to break up rouleaux. Rouleaux formation rarely occurs in old stored serum but it tends to be increased rather than diminished by inactivation of the serum. A positive differentiation from agglutination occasionally is quite difficult.

In rare instances a serum may cause some non-

specific clumping of red cells if the mixture is kept at ice-box temperature.[8] This phenomenon is commonly known as "auto-agglutination," since the cells of the same individual are agglutinated, as well as those of other individuals, quite regardless of their group. The significance of the phenomenon is not known. The degree of clumping is usually slight, and it breaks up promptly when the preparation is warmed to body temperature. Possible confusion may be avoided by warming the preparations in this way before examination.

[8] Clough, M. C., and Richter, I. M. "Study of an auto-agglutinin occurring in a human serum." *Bulletin Johns Hopkins Hosp.*, 1918, xxix, 21.

THE END

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